(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 September 2004 (23.09.2004)

PCT

(10) International Publication Number
WO 2004/080466 A1

(51) International Patent Classification[7]: A61K 31/522, 31/519, 31/7068, C07H 19/14, 19/16, 19/20, 19/173, 19/23, C07F 9/6561, C07D 473/28

(21) International Application Number: PCT/US2003/006992

(22) International Filing Date: 7 March 2003 (07.03.2003)

(25) Filing Language: English

(26) Publication Language: English

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK (utility model), SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *with international search report*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

# (54) Title: CYTIDINE ANALOGS AND METHODS OF USE

(57) Abstract: Cytidine analogs, their prodrugs and/or metabolites are employed as pharmaceutically active compounds for treatment of diseases responsive to such compounds. Particularly preferred diseases include viral diseases (e.g., HCV infection) and neoplasms.

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# CYTIDINE ANALOGS AND METHODS OF USE

## Field of The Invention

The field of the invention is nucleoside analogs, and particularly cytidine analogs and their therapeutic use.

## Background of The Invention

Nucleosides and related compounds interact with many biological targets, and some nucleoside analogues have been used as antimetabolites for treatment of cancers and viral infections. After entry into the cell, many nucleoside analogues can be phosphorylated to monophosphates by nucleoside kinases, and then further phosphorylated by nucleoside monophosphate kinases and nucleoside diphosphate kinases to give nucleoside triphosphates. Once a nucleoside analogue is converted to its triphosphate inside the cell, it can be incorporated into DNA or RNA. Incorporation of certain unnatural nucleoside analogues into nucleic acid replicates or transcripts can interrupt gene expression by early chain termination, or by interfering with the function of the modified nucleic acids. In addition, certain nucleoside analogue triphosphates are very potent, competitive inhibitors of DNA or RNA polymerases, which can significantly reduce the rate at which the natural nucleoside can be incorporated. Many anti-HIV nucleoside analogues fall into this category, including 3'-*C*-azido-3'-deoxythymidine, 2',3'-dideoxycytidine, 2',3'-dideoxyinosine, and 2',3'-didehydro-2',3'-dideoxythymidine.

Various nucleoside analogues can also act in other ways, for example, causing apoptosis of cancer cells and/or modulating immune systems. In addition to nucleoside antimetabolites, a number of nucleoside analogues that show very potent anticancer and antiviral activities act through still other mechanisms. Some well-known nucleoside anticancer drugs are thymidylate synthase inhibitors such as 5-fluorouridine, and adenosine deaminase inhibitors such as 2-chloroadenosine. A well-studied anticancer compound, neoplanocin A, is an inhibitor of S-adenosylhomocysteine hydrolase, which shows potent anticancer and antiviral activities.

Among various nucleoside and nucleotide analogs, cytidine nucleoside analogs have shown significant antiviral and antineoplastic activity (see *e.g.*, Carbone et al., *Biochem*

*Pharmacol.* 2001 Jul 1;62(1):101-10; or Miura et al., *Jpn. J. Cancer Res.* 2001 May;92(5):562-7; or Christensen et al., *Antiviral Res.* 2000 Nov;48(2):131-42). Many of those cytosine analogs, however, have relatively significant side effects.

Further cytidine nucleoside analogs have been prepared such that an additional ring is fused to the cytidine ring, and such nucleosides were used in various non-pharmaceutical methods. For example, Simmonds et al. describe cytidine analogs that can be employed for labeling of in vitro prepared nucleic acids in WO99/06422 and U.S. Pat. No. 6,444,682. In another example, EP1 225 234 to Sampson, cytidine analogs are employed as substrates in sequencing reactions to reduce formation of secondary structures in *in vitro* transcripts to reduce the error rate of sequencing. Alternatively, some of the cytidine analogs may be employed as fluorescent building blocks in an *in vitro* nucleic acid synthesis to generate fluorescent labeled probes as described in EP 0 235 301 to H. Inoue et al.

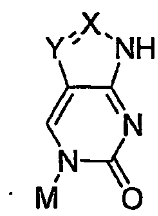
Unfortunately, while numerous cytidine nucleoside analogs are known in the art, all or almost all of them suffer from various disadvantages. Among other problems, many of the known nucleoside analogues that inhibit tumor growth or viral infections are also toxic to normal mammalian cells, primarily because these nucleoside analogues lack adequate selectivity between the normal cells and the virus-infected host cells or cancer cells. Consequently, where cytidine nucleoside analogs are readily incorporated into a nascent nucleic acid, a selective therapeutic function is often not apparent.

Therefore, although various cytidine nucleoside analogs are known in the art, there is still a need to provide new cytidine analogs and methods with improved biological and/or pharmaceutical characteristics.

## Summary of the Invention

The present invention is directed to various cytidine nucleoside analogs, their prodrugs and/or metabolites, and methods of use for contemplated compounds.

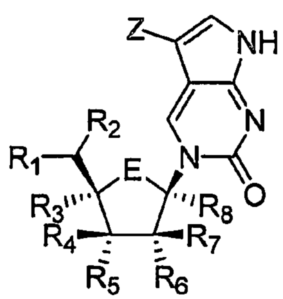
In one aspect of the inventive subject matter, contemplated compounds will have a structure according to **Formula 1** with substituents X, Y, and M as described in the detailed description below, and wherein the compound may be in D- or L-configuration:



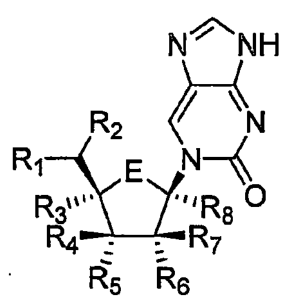
*Formula 1*

Especially preferred compounds according to Formula 1 will further include a phosphate, thiophosphate, phosphonate, or phosphoamidate group covalently coupled to the sugar or sugar analog. Alternatively, or additionally, such compounds may comprise a moiety that increases selectivity of the compound to a target cell or target organ, wherein at least part of the moiety is removed from the compound in the target cell or target organ. Particularly suitable moieties will increase selectivity of the compound to a target cell, wherein at least part of the moiety is removed from the compound in the target cell, and wherein the moiety forms an ester or cyclic diester with the phosphate, thiophosphate, phosphonate, or phosphoamidate group.

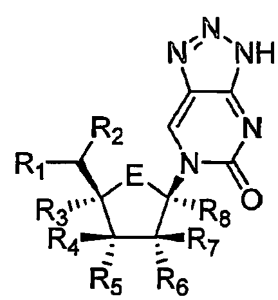
Thus, in another aspect of the inventive subject matter, contemplated compounds include those having a structure according to **Formulae 2-7** in which the substituents X, Y, Z, E, G, $R^1$, $R^2$, $R^3$, $R^4$, $R^5$, $R^6$, $R^7$ and $R^8$ are defined as described in the detailed description below, and wherein the sugar of the compound may be in D- or L-configuration:



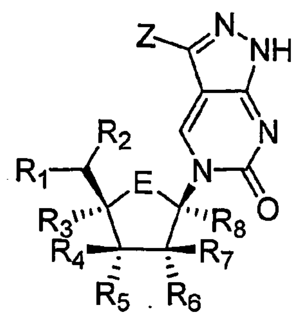
*Formula 2*



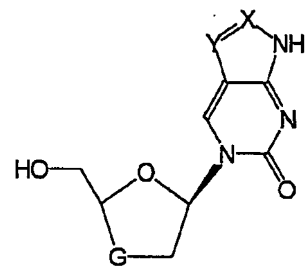
*Formula 3*



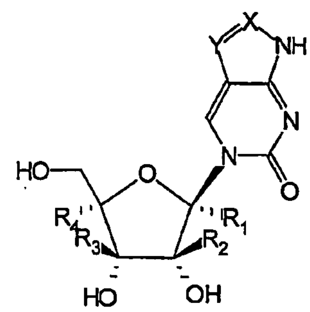
*Formula 4*



*Formula 5*



*Formula 6*



*Formula 7*

In a still further contemplated aspect of the inventive subject matter, a pharmaceutical composition will comprise a compound according to Formula 1 (*supra*) in which the substituents are defined as provided in the respective portion of the detailed description below, and wherein the compound is present at a concentration effective to inhibit propagation of a virus in a patient to which the composition is administered.

In particularly contemplated compositions, the compound will further comprise a phosphate, thiophosphate, phosphonate, or phosphoamidate group covalently coupled to the sugar or sugar analog, and may include a moiety that increases selectivity of the compound to a target cell or target organ, and wherein at least part of the moiety is removed from the compound in the target cell or target organ. Especially contemplated moieties include those in which the moiety forms an ester or cyclic diester with the phosphate, thiophosphate, phosphonate, or phosphoamidate group.

Consequently, the inventors contemplate a method of treating a viral infection in a patient in which a compound according to Formula 1 (*supra*, with substituents as defined in the respective portion of the detailed description below) is administered to the patient at a dosage effective to reduce viral propagation in the patient. Particularly contemplated virus infections include infections with a virus belonging to the family of *Flaviviridae*, and especially HCV virus infections.

Thus, a method of inhibiting a viral polymerase may include a step in which the viral polymerase is presented with a compound according to Formula 1 (*supra*, with substituents as defined in the respective portion of the detailed description below) at a concentration effective to inhibit the viral polymerase. In both, the method of treating a viral infection as well as the method of inhibiting a viral polymerase, the step of presenting may

further comprise conversion of a prodrug of the compound according to Formula 1 into the compound according to Formula 1, and/or conversion of the compound according to Formula 1 into a metabolite of the compound according to Formula 1.

Various objects, features, aspects and advantages of the present invention will become more apparent from the following detailed description of preferred embodiments of the invention.

## Brief Description of The Drawing

Figure 1A is an autoradiograph of a gel with the reaction products of an incubation of a first set of nucleotides and a first template with HCV-NS5B.

Figure 1B is an autoradiograph of a gel with the reaction products of an incubation of a second set of nucleotides and a second template with HCV-NS5B.

## Detailed Description

The inventors discovered that various cytidine analogs, their prodrugs, and/or metabolites exhibit desirable pharmacological properties, and may be particularly useful as antiviral agents and/or antineoplastic agents. The inventors particularly contemplate cytidine analogs in which an additional nitrogen-containing ring is fused to the pyrimidine ring of a cytidine. Consequently, the inventors contemplate in further preferred aspects, pharmaceutical compositions comprising contemplated compounds, methods of treating a viral infection using contemplated compounds, and methods of inhibiting a viral polymerase using contemplated compounds.

The term "alkyl" as used herein includes all saturated hydrocarbon groups in a straight, branched, or cyclic configuration (also referred to as cycloalkyl, see below), and particularly contemplated alkyl groups include lower alkyl groups (*i.e.*, those having six or less carbon atoms). Exemplary alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, secbutyl, tertiary butyl, pentyl, isopentyl, hexyl, isohexyl, etc. The term "alkenyl" as used herein refers to an alkyl as defined above having at least one double bond. Thus, particularly contemplated alkenyl groups include straight, branched, or cyclic alkene groups having two to six carbon atoms (*e.g.*, ethenyl, propenyl, butenyl, pentenyl, etc.). Similarly, the term "alkynyl" as used herein refers an alkyl or alkenyl as defined above having at least one triple bond, and especially contemplated alkynyls include straight, branched, or cyclic

alkynes having two to six total carbon atoms (*e.g.*, ethynyl, propynyl, butynyl, pentynyl, etc.).

The term "cycloalkyl" as used herein refers to a cyclic alkane (*i.e.*, in which a chain of carbon atoms of a hydrocarbon forms a ring), preferably including three to eight carbon atoms. Thus, exemplary cycloalkanes include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Contemplated cycloalkyls may further include one or more double and/or triple bonds, which may be conjugated. The term "aryl" as used herein refers to an aromatic carbon atom-containing ring, which may further include one or more non-carbon atoms. Thus, contemplated aryl groups include cycloalkenes (*e.g.*, phenyl, naphthyl, etc.) and pyridyl.

The term "alkoxy" as used herein refers to straight or branched chain alkoxides, wherein the hydrocarbon portion may have any number of carbon atoms (and may further include a double or triple bond). For example, suitable alkoxy groups include methoxy (MeO-), ethoxy, isopropoxy, etc. Similarly, the term "alkylthio" refers to straight or branched chain alkylsulfides, wherein the hydrocarbon portion may have any number of carbon atoms (and may further include a double or triple bond). For example, contemplated alkylthio groups include methylthio (MeS-), ethylthio, isopropylthio, etc. Likewise, the term "alkylamino" refers to straight or branched alkylamines, wherein the hydrocarbon portion may have any number of carbon atoms (and may further include a double or triple bond). Furthermore, the remaining hydrogen of the amino group in the alkylamino group may be substituted with another alkyl group (or other subtituent). Therefore, exemplary alkylamino groups include methylamino, dimethylamino, ethylamino, diethylamino, isopropylamino, t-butylamino, etc. Still further, the term "alkylsulfonyl" refers to straight or branched chain alkylsulfones, wherein the hydrocarbon portion may have any number of carbon atoms (and may further include a double or triple bond). For example, contemplated alkylsulfonyl groups include methylsulfonyl ($MeSO_2$-), ethylsulfonyl, isopropylsulfonyl, etc.

The term "alkyloxycarbonyl" as used herein refers to straight or branched chain esters of a carboxylic acid (derivative) and may have any number of carbon atoms (and may still further include a double or triple bond). Exemplary alkyloxycarbonyl groups include methyloxycarbonyl (MeOCO--), ethyloxycarbonyl, and butyloxycarbonyl.

The term "halogen" as used herein refers to fluorine, chlorine, bromine and iodine.

The term "substituted" as used herein refers to a replacement of an atom or chemical group (*e.g.*, H, $NH_2$, or OH) with a functional group, and particularly contemplated functional groups include nucleophilic groups (*e.g.*, $-NH_2$, -OH, -SH, -NC, etc.), electrophilic groups (*e.g.*, C(O)OR, C(X)OH, etc.), polar groups (*e.g.*, -OH, C(O)Cl, etc.), non-polar groups (*e.g.*, aryl, alkyl, alkenyl, alkynyl, etc.), ionic groups (e.g., $-NH_3^+$), halogens (e.g., -F, -Cl), and all chemically reasonable combinations thereof. Moreover, the term "substituted" also includes multiple degrees of substitution, and where multiple substituents are disclosed or claimed, the substituted compound can be independently substituted by one or more of the disclosed or claimed substituent moieties.

The term "functional group" and "substituent" are used interchangeably herein and refer to groups including nucleophilic groups (*e.g.*, $-NH_2$, -OH, -SH, -NC, -CN etc.), electrophilic groups (*e.g.*, C(O)OR, C(X)OH, C(Halogen)OR, etc.), polar groups (*e.g.*, -OH), non-polar groups (*e.g.*, aryl, alkyl, alkenyl, alkynyl, etc.), ionic groups (e.g., $-NH_3^+$), and halogens.

As also used herein, the terms "heterocycle", "cycloheteroalkyl", and "heterocyclic base" are used interchangeably herein and refer to any compound in which a plurality of atoms form a ring via a plurality of covalent bonds, wherein the ring includes at least one atom other than a carbon atom. Particularly contemplated heterocyclic bases include 5- and 6-membered rings with nitrogen, sulfur, and/or oxygen as the non-carbon atom (*e.g.*, imidazole, pyrrole, triazole, dihydropyrimidine). Further contemplated heterocylces may be fused (*i.e.*, covalently bound) to another ring or heterocycle, and are thus termed "fused heterocycle" or "fused heterocyclic base" herein.

Especially contemplated fused heterocycles include a 5-membered ring fused to a 6-membered ring (*e.g.*, purine, pyrrolo[2,3-d]pyrimidine), and a 6-membered ring fused to another 6-membered or higher ring (*e.g.*, pyrido[4,5-d]pyrimidine, benzodiazepine). Examples of these and further preferred heterocyclic bases are given below. Still further contemplated heterocyclic bases may be aromatic, or may include one or more double or triple bonds. Moreover, contemplated heterocyclic bases and fused heterocycles may be substituted in one or more positions.

As further used herein, the term "sugar" refers to all carbohydrates having the general formula $C_nH_{2n}O_2$ (with n typically being an integer in the rage of 2-10) and

derivatives thereof, wherein particularly contemplated derivatives include deletion, substitution or addition of a chemical group or atom in the sugar. For example, especially contemplated deletions include 2'-deoxy and/or 3'-deoxy sugars. Especially contemplated substitutions include replacement of the ring-oxygen with sulfur or methylene, or replacement of a hydroxyl group with a halogen, an amino-, sulfhydryl-, or methyl group, and especially contemplated additions include methylene phosphonate groups. Further contemplated sugars also include sugar analogs (*i.e.*, not naturally occurring sugars), and particularly carbocyclic ring systems. The term " carbocyclic ring system" as used herein refers to any molecule in which a plurality of carbon atoms form a ring, and in especially contemplated carbocyclic ring systems the ring is formed from 3, 4, 5, or 6 carbon atoms. Examples of these and further preferred sugars are given below.

The term "nucleoside" refers to all compounds in which a heterocyclic base is covalently coupled to a sugar, and an especially preferred coupling of the nucleoside to the sugar includes a C1'-(glycosidic) bond of a carbon atom in a sugar to a carbon- or heteroatom (typically nitrogen) in the heterocyclic base. The term "nucleoside analog" as used herein refers to all nucleosides in which the sugar is not a ribofuranose and/or in which the heterocyclic base is not a naturally occurring base (*e.g.*, A, G, C, T, I, etc.). Similarly, the term "nucleotide" refers to a nucleoside to which a phosphate group is coupled to the sugar. Likewise, the term "nucleotide analog" refers to a nucleoside analog to which a phosphate group (or modified phosphate group) is coupled to the sugar.

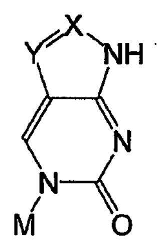
The term "pharmaceutical composition" as used herein refers to a product comprising at least one of the contemplated compounds and an inert ingredient or inert ingredients that make up a carrier. Consequently, contemplated pharmaceutical compositions especially include those made by admixing contemplated compounds according to the inventive subject matter and a pharmaceutically acceptable carrier.

The terms "administration of" and "administering a" compound as used herein refers to providing the compound (which may also be in a prodrug form or in a metabolite form) to an individual in need of such compound. Most typically, contemplated compounds will be orally (*e.g.*, via tablet, syrup, etc.) or parenterally (*e.g.*, injection, transdermal delivery, etc.) administered. Similarly, the term "presenting" a polymerase with contemplated compounds as used herein refers to providing the compound (which may also be in a prodrug form or in a metabolite form) to the polymerase, and will most typically

include contacting the polymerase with the contemplated compound (which may also be in a prodrug- or metabolite form). For example, direct contact may be achieved by admixing or adding contemplated compounds to a fluid in which the polymerase is present; while indirect contact may be achieved by admixing or adding contemplated compounds to a cell or organ in which the polymerase is present.

**Contemplated Compounds**

In one aspect of the inventive subject matter, contemplated compounds have a structure according to **Formula 1**



*Formula 1*

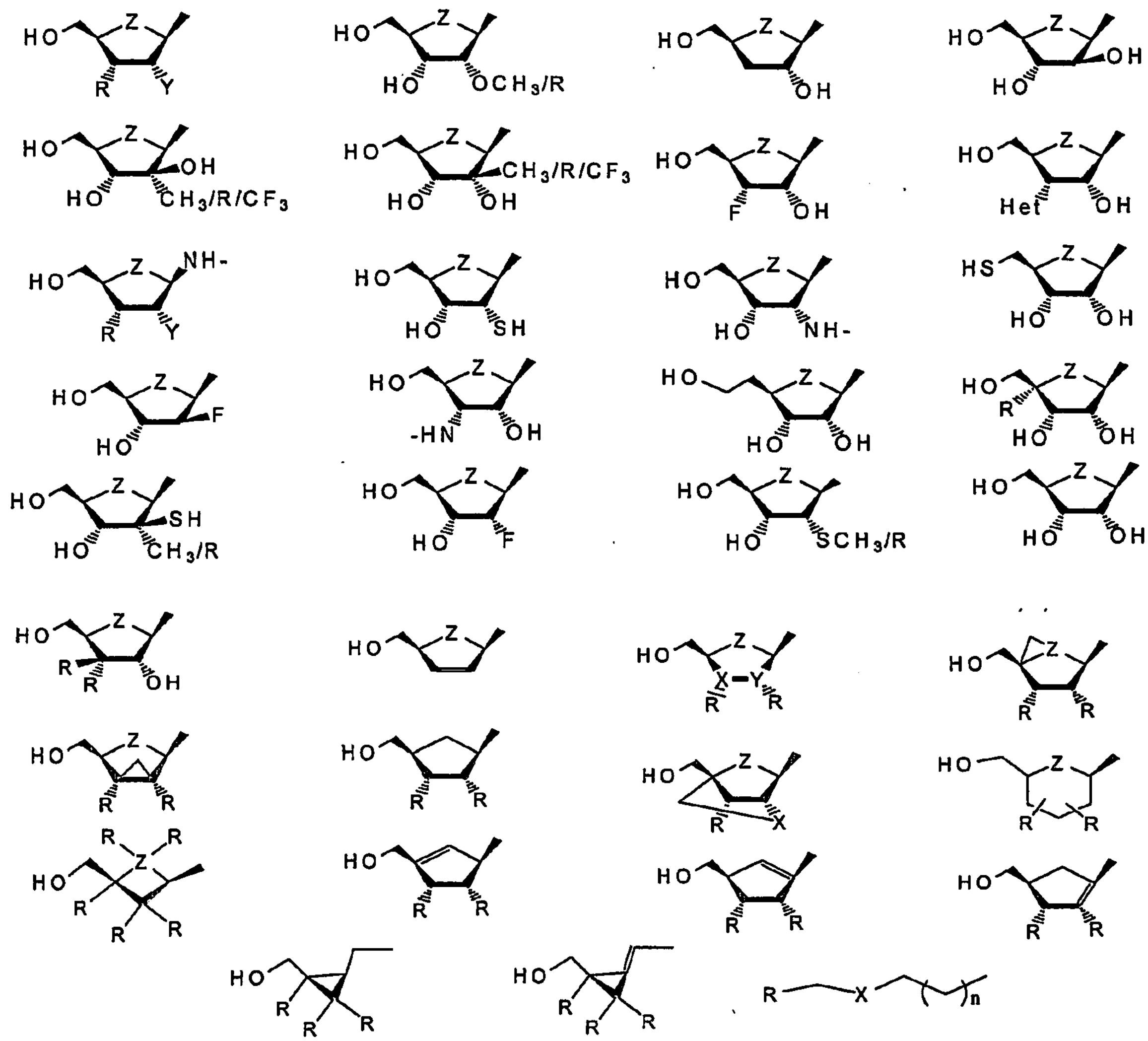
in which -X=Y- is -N=N-, -CH=N-, -N=CZ- or -CH=CZ-, wherein Z is H, halogen, or alkyl, wherein M is a sugar or sugar analog, and wherein the compound has a D-configuration or an L-configuration. In one sub-aspect of such compounds, contemplated compounds may exclude those where M is a sugar with a ribofuranose ring having a heteroatom and substituents $R_1$ and $R_2$ on the C3'-atom, $R_3$ and $R_4$ on the C2'-atom, and $R_5$ on the C5'-atom, wherein $R_1$, $R_2$, $R_3$, and $R_4$ together are not independently H, OH, F, $NH_2$, $N_3$, O-hydrocarbyl, or a reporter moiety, when the heteroatom is O, S, Se, SO, N-alkyl, or $CH_2$, and when $R_5$ is OH, SH, $NH_2$, monophosphate, diphosphate, triphosphate, thiophosphate, or boranophosphate. Alternatively, or additionally, contemplated compounds may also exclude those in which M is a sugar comprising a cyclopropenyl group or a morpholino group, or in which M is a phosphonylmethoxyethyl group.

With respect to the sugar, it should be recognized that all sugars and sugar analogs are suitable for use herein. Consequently, it is contemplated that particularly suitable sugars will have a general formula of $C_nH_{2n}O_n$, wherein n is between 2 and 8, and wherein (where applicable) the sugar is in the D- or L-configuration. Moreover, it should be appreciated that there are numerous equivalent modifications of such sugars known in the art (e.g., sugar

analogs), and all of such modifications are specifically included herein. For example, some contemplated alternative sugars will include sugars in which the heteroatom in the cyclic portion of the sugar is an atom other than oxygen (*e.g.*, sulfur, carbon, or nitrogen) analogs, while other alternative sugars may not be cyclic but in a linear (open-chain) form. Suitable sugars may also include one or more double bonds.

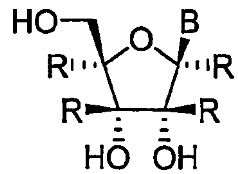
Still further specifically contemplated alternative sugars include those with one or more non-hydroxyl substituents, and particularly contemplated substituents include mono-, di-, and triphosphates (preferably as $C_5$' esters), alkyl groups, alkoxygroups, halogens, amino groups and amines, sulfur-containing substituents, etc. It is still further contemplated that all contemplated substituents (hydroxyl substituents and non-hydroxyl substituents) may be directed in the alpha or beta position.

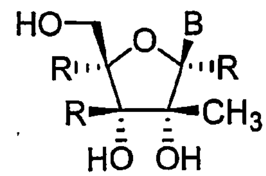
Numerous contemplated sugars and sugar analogs are commercially available. However, where contemplated sugars are not commercially available, it should be recognized that there are various methods known in the art to synthesize such sugars. For example, suitable protocols can be found in "Modern Methods in Carbohydrate Synthesis" by Shaheer H. Khan (Gordon & Breach Science Pub; ISBN: 3718659212), in U.S. Pat Nos. 4,880,782 and 3,817,982, in WO88/00050, or in EP199,451. An exemplary collection of further contemplated sugars and sugar analogs is depicted below, wherein all of the exemplary sugars may be in D- or L-configuration, and wherein at least one of the substituents (typically H or OH) on the $C_1$'-$C_5$' atom of the sugar may be in either alpha or beta orientation.



X, Y, Z = O, S, Se, NH, NR, $CH_2$, CHR, P(O), P(O)OR
R = H, OH, NHR, halo, $CH_2OH$, COOH, $N_3$, alkyl, aryl, alkynyl, heterocycles, OR, SR, $P(O)(OR)_2$ OCOR, NHCOR, $NHSO_2R$, $NH_2NH_2$, amidine, substituted amidine, quanidine, substituted gyanidine

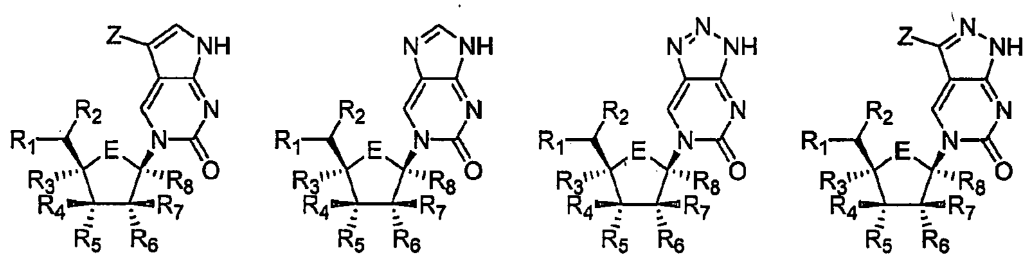
An especially contemplated class of sugars comprises alkylated sugars, wherein one or more alkyl groups (or other groups, including alkenyl, alkynyl, aryl, halogen, $CF_3$, $CHF_2$, $CCl_3$, $CHCl_2$, $N_3$, $NH_2$, etc.) are covalently bound to sugar at the $C'_1$, $C'_2$,$C'_3$,$C'_4$, and/or $C'_5$ atom. In such alkylated sugars, it is especially preferred that the sugar portion comprises a furanose (most preferably a D- or L-ribofuranose), and that at least one of the alkyl groups is a methyl group. Of course, it should be recognized that the alkyl group may or may not be substituted with one or more substituents. One exemplary class of preferred sugars is depicted below:





in which B is hydrogen, hydroxyl, or a heterocyclic base (see below), R is independently hydrogen, hydroxyl, substituted or unsubstituted alkyl (branched, linear, or cyclic), with R including between one and twenty carbon atoms.

Consequently, and especially where preferred compounds according to Formula 1 include a sugar in a furanose configuration, contemplated compounds will have a structure according to any one of Formula 2, Formula 3, Formula 4, and Formula 5

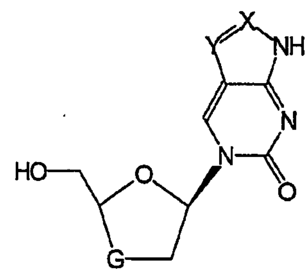


*Formula 2* *Formula 3* *Formula 4* *Formula 5*

wherein $R_1$ is H, OH, O(monophosphate), O(diphosphate), O(triphosphate), O(acyl), O(alkyl), O(alkenyl), S(alkyl), S(O)(alkyl), S(O)(O)(alkyl), phosphonate, $CH_2$(phosphonate), $CF_3$, $CHF_2$, $CH_2F$, $CH_2OH$, $CH_2O$(alkyl), halogen, $NO_2$, $NH_2$, NH(alkyl), NH(acyl), N(lower alkyl)$_2$, or N(acyl)$_2$, and where $R_1$ comprises a phosphate or phosphonate, the phosphate or phosphonate is optionally stabilized or masked; $R_2$, $R_3$, $R_4$, $R_7$ and $R_8$ are independently H, OH, O(acyl), O(alkyl), O(alkenyl), O(alkynyl), S(alkyl), S(O)(alkyl), S(O)(O)(alkyl), alkyl, alkenyl, alkynyl, cyano, $CF_3$, $CHF_2$, $CH_2F$, $CH_2OH$, $CH_2O$(alkyl), Br-vinyl, 2-Br-ethyl, C(O)O(alkyl), halogen, azido, $NO_2$, $NH_2$, NH(alkyl), -NH(acyl), -N(alkyl)$_2$, or N(acyl)$_2$; $R_5$ and $R_6$ are independently H, OH, O(acyl), O(alkyl), O(alkenyl), S(alkyl), S(O)(alkyl), S(O)(O)(alkyl), alkyl, alkenyl, alkynyl, cyano, $CF_3$, $CHF_2$, $CH_2F$, $CH_2OH$, $CH_2O$(alkyl), Br-vinyl, 2-Br-ethyl, C(O)O(alkyl), halogen, azido, $NO_2$, $NH_2$, NH(alkyl), NH(acyl), N(alkyl)$_2$, N(acyl)$_2$; wherein E is O, S, $SO_2$, C(=$CH_2$), $NR_6$, or $CH_2$; wherein optionally one of $R_4$ and $R_5$ is null, then the other of $R_4$ and $R_5$ is coupled to the sugar via a double bond; optionally one of $R_6$ and $R_7$ is null, then the other of $R_6$ and $R_7$ is coupled to the sugar via a double bond; and optionally $R_5$ and $R_6$ are null, then

the C2'-atom and C3'-atom of the sugar are coupled to each other via a double bond (wherein the sugar may be in D- or L-configuration).

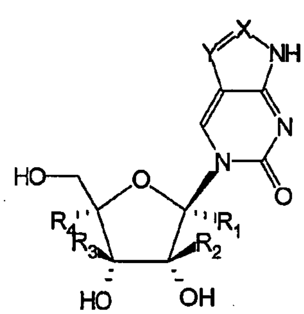
Alternatively, and especially where particularly preferred compounds include a sugar in furanose configuration in which the sugar has a heteroatom in C3'-position, contemplated compounds will have a structure according to Formula 6



*Formula 6*

wherein -X=Y- is -N=N-, -CH=N-, -N=CZ- or -CH=CZ-, wherein Z is H, halogen, or alkyl; and wherein G is S, O $CH_2$, or CHOH, and wherein the compound is in D- or L-configuration.

In still further contemplated aspects, and especially where the sugar has a furanose configuration and at least one of the C1'-, C2'-, C3'-, and C4'-position in the sugar is substituted (*i.e.*, has a substituent other than H), contemplated compounds will have a structure according to Formula 7



*Formula 7*

wherein -X=Y- is -N=N-, -CH=N-, -N=CZ or -CH=CZ-, wherein Z is H, halogen, or alkyl; wherein one of $R_1$, $R_2$, $R_3$, and $R_4$ is an alkyl, and the remaining of $R_1$, $R_2$, $R_3$, and $R_4$

is H; wherein the compound is in an L- or D-configuration, with the proviso that not all of $R_1$, $R_2$, $R_3$, and $R_4$ are H.

In yet another aspect, it should be appreciated that the compounds according to the inventive subject matter may also be in a prodrug form. Suitable prodrug forms are preferably (but not necessarily) less active than the corresponding non-prodrug form of contemplated compounds, and may be converted to the corresponding non-prodrug form in one or more than one step. For example, conversion from the prodrug form into the corresponding non-prodrug form may occur intracellularly (*e.g.*, enzymatically or via reduction in non-enzymatic redox reaction) or extracellularly, in a single step or multiple steps.

However, especially preferred prodrug forms include those that confer a particular specificity towards a diseased or infected cell or organ, and exemplary contemplated prodrug forms are described in "Prodrugs" by Kenneth B. Sloan (Marcel Dekker; ISBN:0824786297), "Design of Prodrugs" by Hans Bundgaard (ASIN: 044480675X), or in copending U.S. application number 09/594410, filed 06/16/2000, all of which are incorporated by reference herein. Further suitable prodrug forms of contemplated compounds may include a moiety that is covalently coupled to at least one of the C2'-OH, C3'-OH, and C5'-OH (or phosphate, phosphonate, phosphorothioate, or boranophosphate esters with the C2'-OH, C3'-OH, and C5'-OH), wherein at least part of the moiety is preferentially cleaved from the compound in a target cell (*e.g.*, Hepatocyte) or a target organ (*e.g.*, liver). While not limiting to the inventive subject matter, it is preferred that the prodrug is converted into the active form by a cellular enzyme, and particularly by a cytochrome-associated enzyme system (*e.g.*, CYP-system).

Especially contemplated prodrugs comprise a cyclic phosphate, cyclic phosphonate and/or cyclic phosphoamidates, which are preferentially cleaved in a hepatocyte to produce the phosphorylated forms of contemplated compounds (which may be further phosphorylated intracellularly via a kinase). There are numerous such prodrugs known in the art, and all of those are considered suitable for use herein. However, especially contemplated prodrug forms are disclosed in WO 01/47935 (Novel Bisamidate Phosphonate Prodrugs), WO 01/18013 (Prodrugs For Liver Specific Drug Delivery), WO 00/52015 (Novel Phosphorus-Containing Prodrugs ), and WO 99/45016 (Novel Prodrugs For Phosphorus-Containing Compounds), all of which are incorporated by reference herein.

Consequently, especially suitable prodrug forms include those targeting a hepatocyte or the liver.

Still further particularly preferred prodrugs include those described by Renze et al. in Nucleosides Nucleotides Nucleic Acids 2001 Apr-Jul;20(4-7):931-4, by Balzarini et al. in Mol Pharmacol 2000 Nov;58(5):928-35, or in U.S. Pat. No. 6,312,662 to Erion et al., U.S. Pat. No. 6,271,212 to Chu et al., U.S. Pat. No. 6,207,648 to Chen et al., U.S. Pat. No. 6,166,089 and U.S. Pat. No. 6,077,837 to Kozak, U.S. Pat. No. 5,728,684 to Chen, and published U.S. Application with the number 20020052345 to Erion, all of which are incorporated by reference herein. Alternative contemplated prodrugs include those comprising a phosphate and/or phosphonate non-cyclic ester, and an exemplary collection of suitable prodrugs is described in U.S. Pat. No. 6,339,154 to Shepard et al., U.S. Pat. No. 6,352,991 to Zemlicka et al., and U.S. Pat. No. 6,348,587 to Schinazi et al., all of which are incorporated by reference herein. Still further particularly contemplated prodrug forms are described in FASEB J. 2000 Sep;14(12):1784-92, Pharm. Res. 1999, Aug 16:8 1179-1185, and Antimicrob Agents Chemother 2000, Mar 44:3 477-483, which are incorporated by reference herein.

Therefore, contemplated compounds may also include a phosphate, thiophosphate, phosphonate, boranophosphate, or phosphoamidate group covalently coupled to the sugar or sugar analog (preferably via a C5'-OH group and thus forming the corresponding ester), wherein such groups may further comprise a moiety that increases selectivity of the compound to a target cell (*e.g.*, virus infected cell, neoplastic cell, or organ-specific cell such as a hepatocyte). Particularly preferred moieties are at least partially removed from contemplated compounds in the target cell. Thus, contemplated compounds may comprise a pivaloyl group or an S-acyl-thioethyl group (*e.g.*, the moiety may form an ester or cyclic diester with the phosphate, thiophosphate, phosphonate, or phosphoamidate group of the compound).

Moreover, it should also be recognized that contemplated compounds also include metabolites of contemplated compounds (which may be converted *in vivo, e.g.*, within a cell or within a living organism, or synthetically produced) to form a pharmaceutically active compound. For example, where contemplated compounds include a C5'-OH group, a intracellular kinase may phosphorylate the C5'-OH group to form the corresponding monophosphate (nucleotide), which may in turn be still further phosphorylated to the

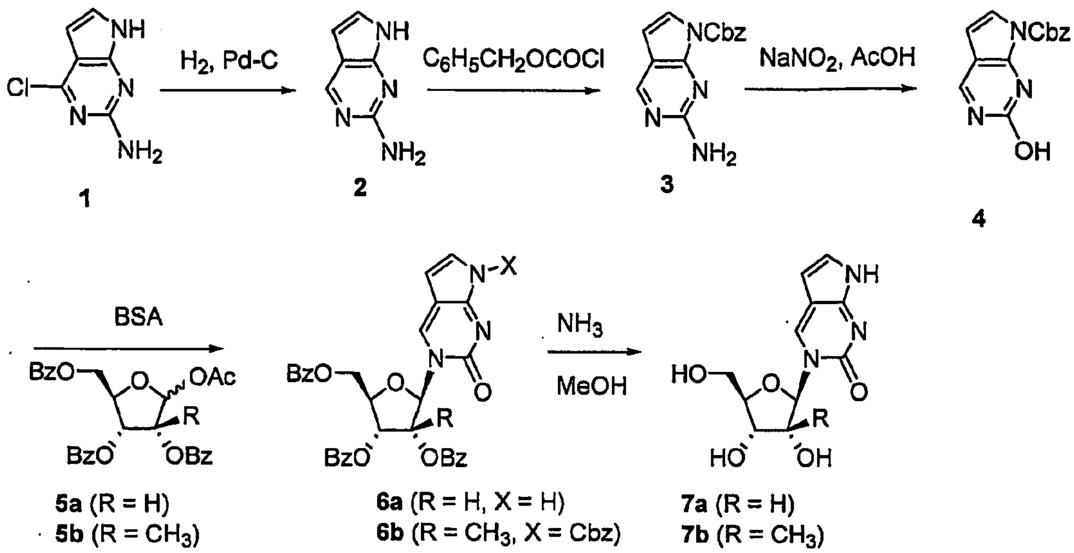
corresponding di- and/or triphosphate (nucleotide). Such phosphorylated compounds may then be the pharmaceutically (more) active form of contemplated compounds. Alternatively, contemplated compounds may also be metabolized to the corresponding aglycon (*i.e.*, heterocyclic base without the sugar portion), or contemplated compounds may undergo various enzymatic degradation (*e.g.*, deaminase reaction, oxidoreductase reaction, etc.) or addition reactions (*e.g.*, amino transferase reaction, kinase reaction, etc.).

## Synthesis of Contemplated Compounds

Contemplated compounds may be synthesized in a variety of procedures, and it should be recognized that the following synthetic schemes are only provided to illustrate exemplary routes through which the compounds according to the inventive subject matter may be obtained. Thus, it should be appreciated that a person of ordinary skill in the art may prepare compounds according to the inventive subject matter in various alternative synthetic procedures.

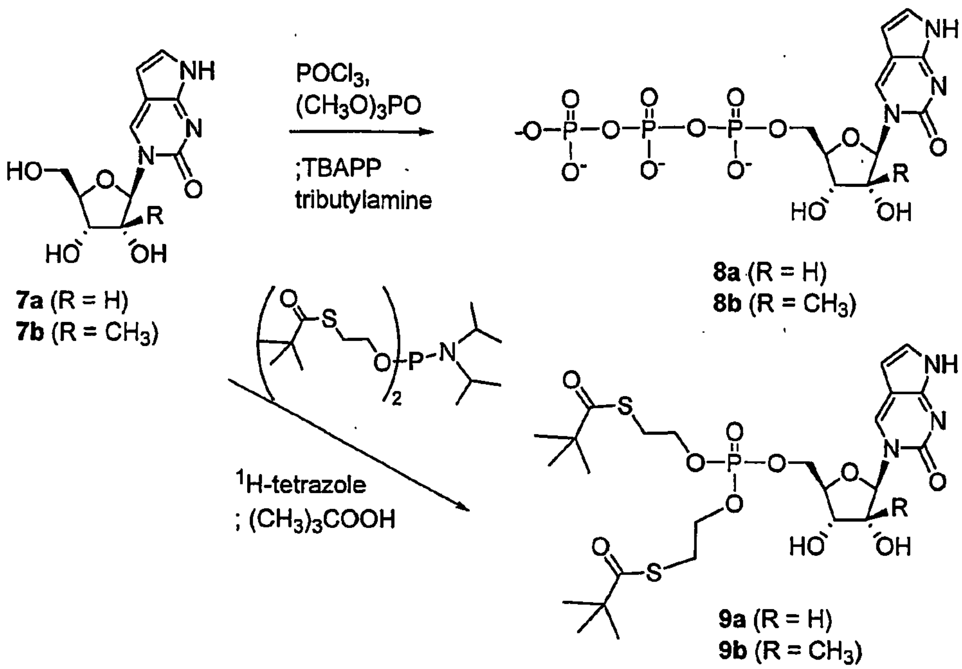
### *Pyrrolo[2,3-d]Pyrimidin-2-one Nucleosides and Corresponding Prodrugs*

Synthesis of pyrrolo[2,3-d]pyrimidin-2-one nucleosides can be achieved following a route depicted in **Scheme 1**. Hydrogenation of commercially available 2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine **1** in the presence of palladium on activated charcoal gives **2**. Protection of pyrrole nitrogen of **2** with benzyloxycarbonyl (Cbz) group is generally desirable for the next diazotization. The diazotization of **3** with sodium nitrite in acetic acid provides the pyrrolopyrimidine **4**. Glycosylation of **4** with two different ribofuranoses **5a** and **5b** yields the corresponding nucleosides **6a** and **6b**, and deprotection of the benzoyl groups of **6a** and **6b** provide the target nucleosides **7a** and **7b**.



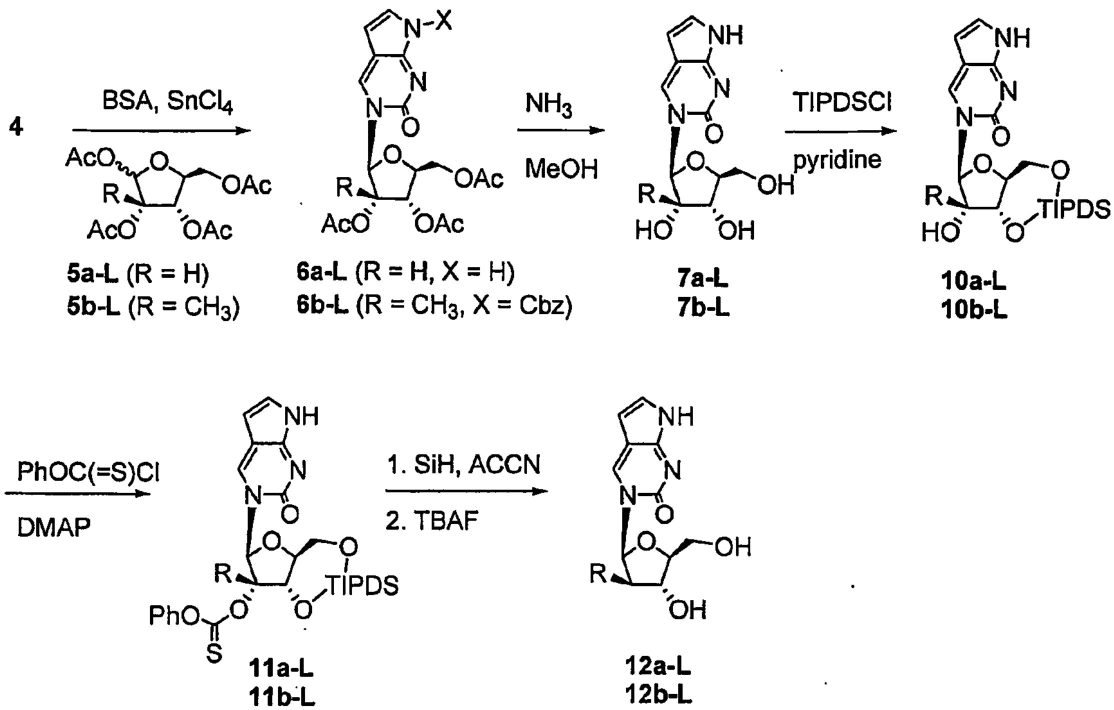

*Scheme 1*

Where derivatives and prodrugs of the compounds according to Scheme 1 are desired, a synthetic route as depicted in **Scheme 2** may be employed. Here, the triphosphates **8a,b** are synthesized from the reaction of **7a,b** with phosphorous oxychloride in trimethylphosphonate, followed by the reaction with tibutylammonium pyrophosphate. Syntheses of prodrugs **9a,b** can be achieved by the treatment of **7a,b** with (*S*-pivaloyl-2-thioethyl)-*N,N*-diisopropylphosphoramidite and the subsequent oxidation with $^{1}H$-tetrazole. Furthermore, substituents on various positions may be incorporated by using a suitably substituted (*e.g.*, alkylated or halogenated) starting material, or by ring formation as described in U.S. Pat. No. 6,444,682 to Simmonds et al., which is incorporated by reference herein.

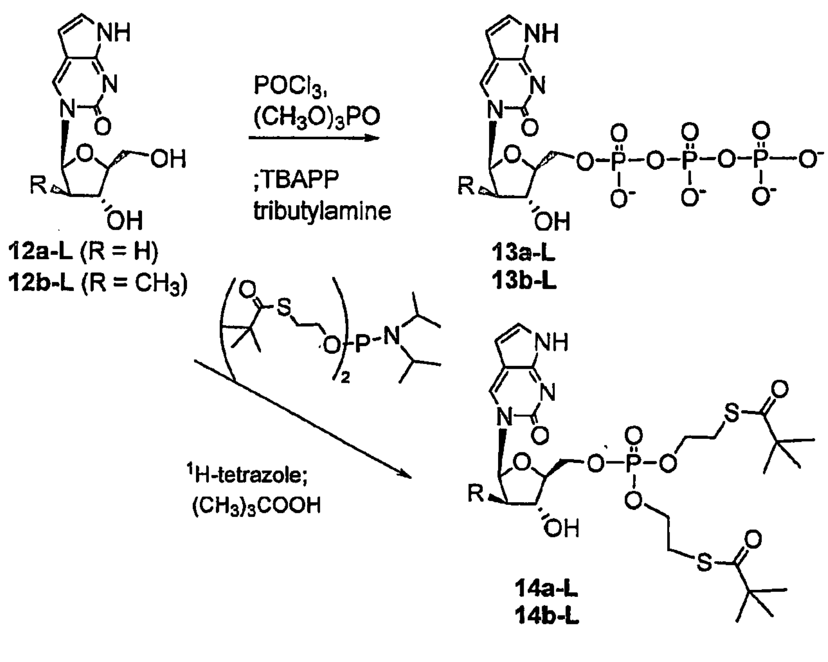



*Scheme 2*

Where L-nucleosides are particularly desired, synthesis may proceed as depicted in **Schemes 3 and 4** (those skilled in the art will recognize that the chemistry depicted for L-nucleosides can also be achieved with D-nucleosides by starting with a D-sugar instead of an L-sugar). Here, base **4** is condensed with the acylated sugar **5a,b-L** in the presence of BSA to yield the protected nucleoside **6a,b-L** which, upon deprotection with methanolic ammonia, leads to **7a,b-L**. Protection of the desired hydroxy groups followed by deoxygenation and deprotection, can yield compounds **12a,b-L**. These nucleosides can be phosphorylated into their triphosphate form or into a prodrug form as shown in Scheme 4.

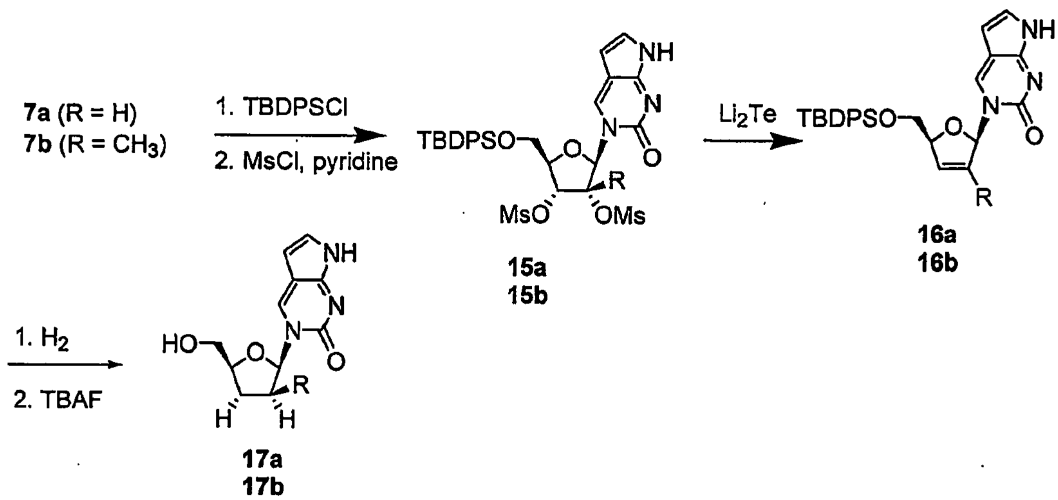



*Scheme 3*



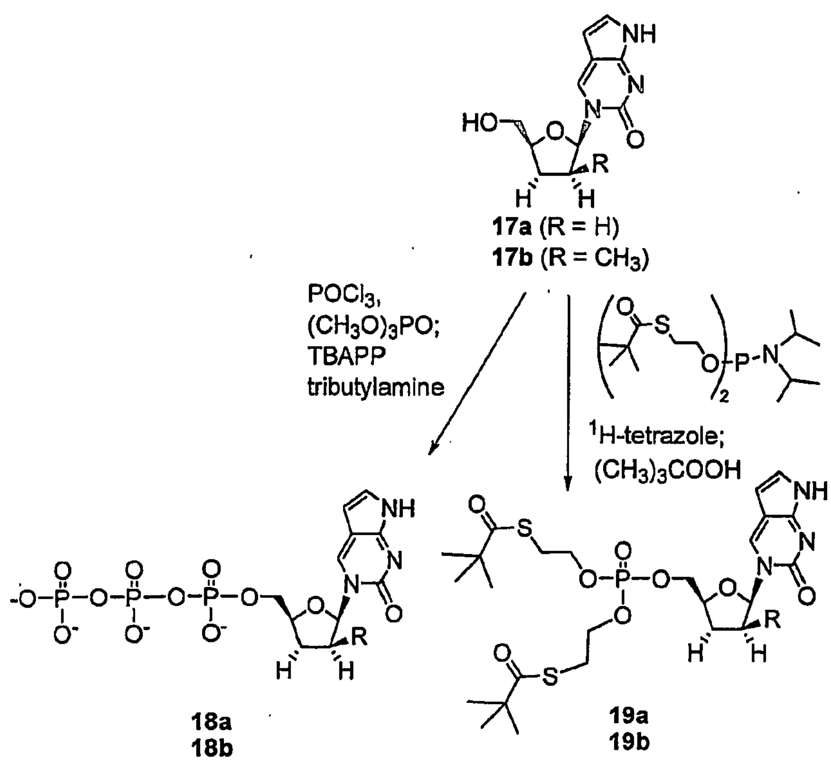

*Scheme 4*

**Scheme 5** shows an example of D-nucleosides, **7a,b,** that can be transformed into the derivatives **17a,b** by a series of protection, elimination, hydrogenation, and deprotection. **16a,b** can also be deprotected to yield their dideoxydidehydro derivatives.




*Scheme 5*

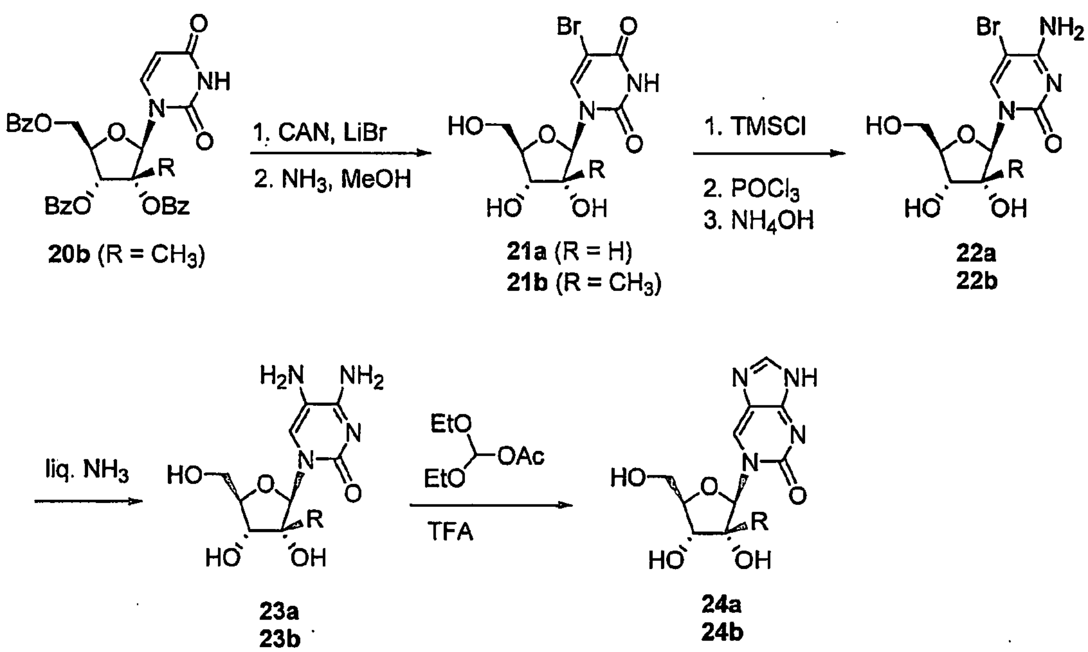
**Scheme 6** depicts the formation of the corresponding SATE prodrugs of the compounds of Scheme 5.



*Scheme 6*

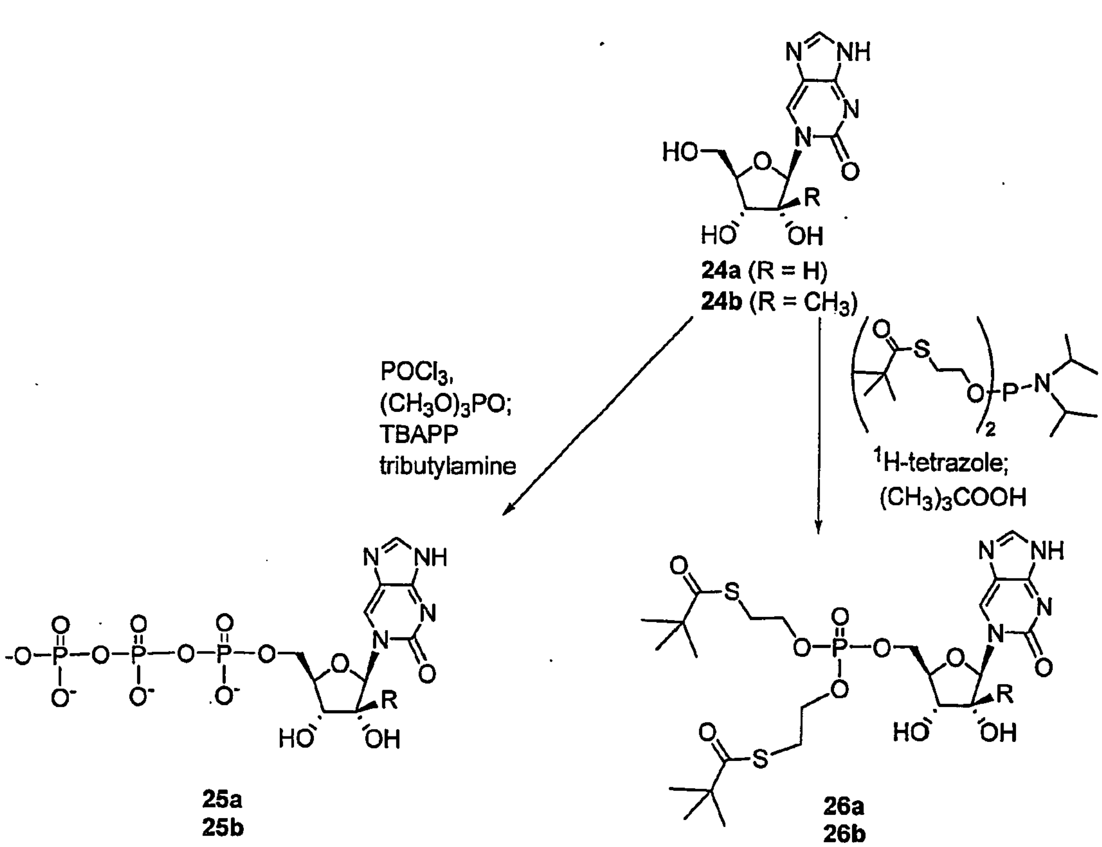
*2-Oxypurine Nucleosides And Corresponding Prodrugs*

In yet a further example, synthesis of various 2-oxypurine nucleosides **24a,b** can be achieved by following a synthetic route as depicted in **Scheme 7**. Here, 5-Bromouridine derivative **21b** is obtained from the reaction of **20b** and lithium bromide in the presence of ammonium cerium(IV) nitrate. Both **21a** and **21b** are transformed to the corresponding 4-amino derivatives **22a,b**. Treatment of **22a,b** with liquid ammonia produces **23a,b**, which is subsequently converted to 2-oxypurine nucleosides **24a,b** by the treatment of diethoxymethyl acetate.




*Scheme 7*

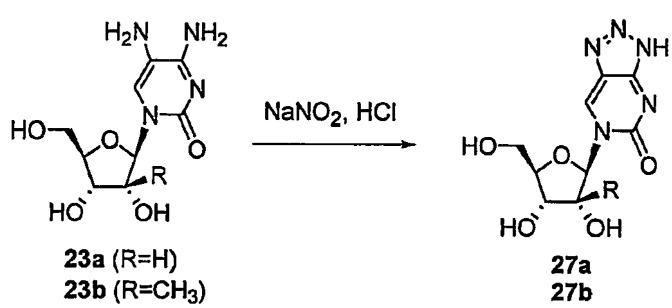
The syntheses of both, the triphosphates and prodrugs of 24 as shown in **Scheme 8** below are achieved by the application of standard protocols described in Scheme 2 above.



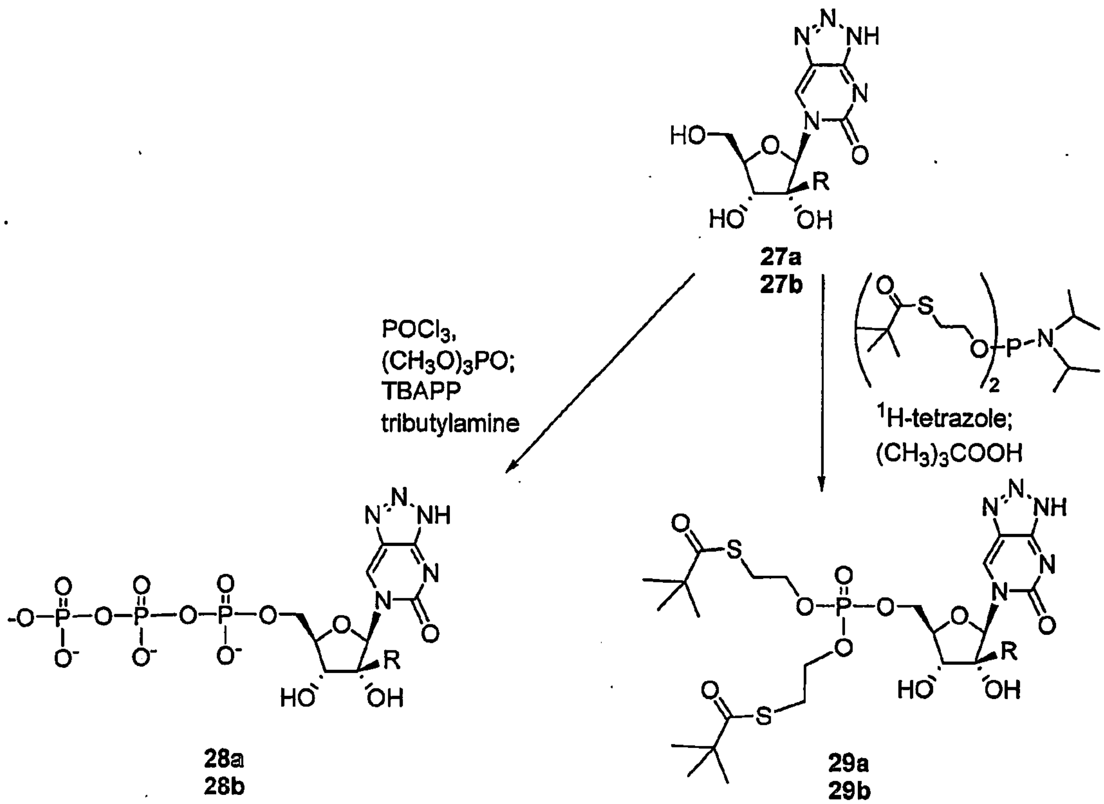

*Scheme 8*

*Triazolopyrimidine Nucleosides And Corresponding Prodrugs*

Syntheses of exemplary triazolopyrimidine nucleosides **27a,b** can be produced from the intermediates **23a,b** by treatment with sodium nitrite as depicted in **Scheme 9** below. Similarly, syntheses of both triphosphates and prodrugs of **27a,b** as depicted in **Scheme 10** are achieved by the application of standard protocols described in Scheme 2.

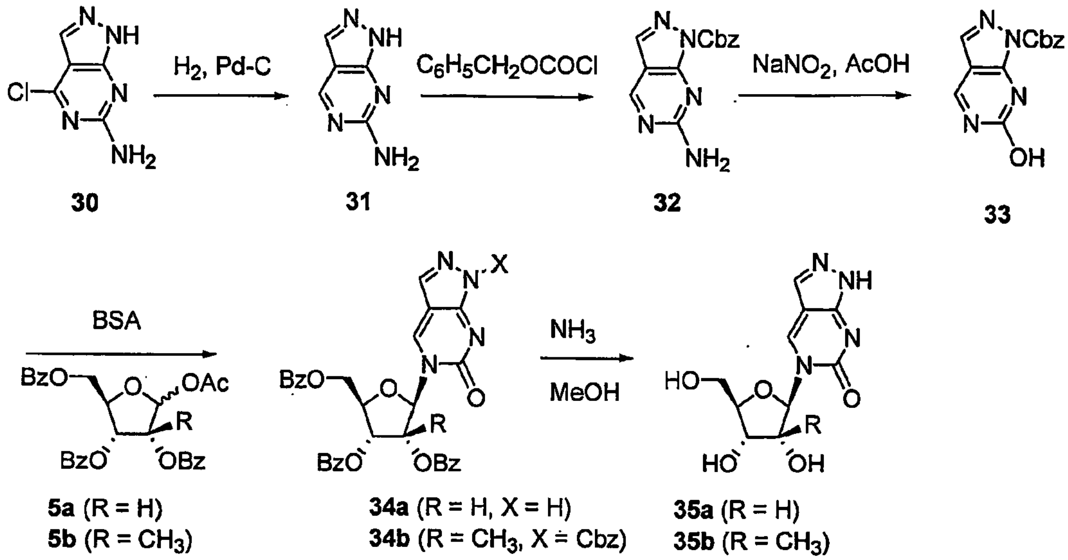



*Scheme 9*



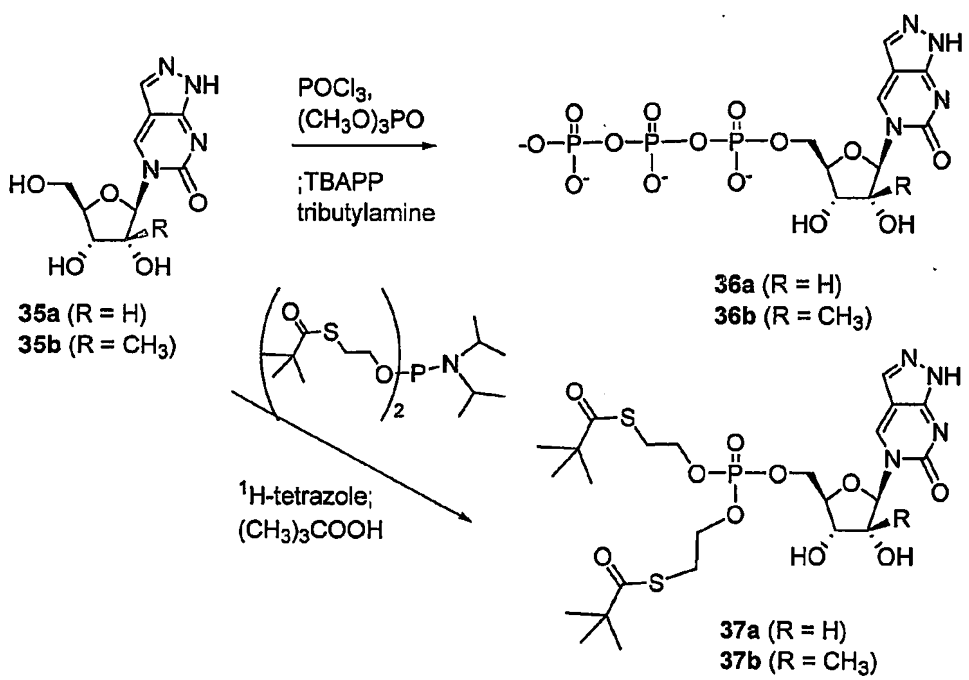

*Scheme 10*

In a particularly preferred aspect, syntheses of pyrazolopyrimidine nucleosides **35a,b** can be achieved as depicted in **Scheme 11.** The conversion of 6-amino-4-chloro-*1H*-pyrazolo[3,4-*d*]pyrimidine (**30,** for synthesis see *e.g.*, Seela, F.; Steker, H., *Helv. Chim. Acta*, **1986**, *69*, 1602-1613) to the target nucleosides **35** is accomplished by application of the same synthetic approach as depicted in Scheme 1. Again, substituents on various positions may be incorporated by using suitably substituted (*e.g.*, alkylated or halogenated) starting material, or by ring formation as described in U.S. Pat. No. 6,444,682 to Simmonds et al., which is incorporated by reference herein.




*Scheme 11*

Standard protocols are again applied for the syntheses of both triphosphates and prodrugs of **36** and are depicted in **Scheme 12** below.




*Scheme 12*

Where contemplated compounds with (unmodified or modified) sugars other than those depicted above are desired, it should be recognized that such sugars may be

synthesized following protocols well known in the art. For example, 2'-deoxy-2',2'-difluoro nucleoside compounds may be prepared as described in Tetrahedron **54** (1998) 3523-3532 (Synthesis of 2-deoxy-3,5-di-O-benzoyl-2,2-difluoro-D-ribose from D-glucose and D-mannose - A formal synthesis of Gemcitabine. Raül Fernández, M. Isabel Matheu, Raouf Echarri and Sergio Castillón.). Alternatively, 2'-deoxy-beta-L-nucleoside compounds may be prepared as described in J. Med. Chem. **43** (2000) 1019-1028 (Monocyclic L-nucleosides with type 1 cytokine-inducing activity. Kanda S. Ramasamy, Robert C. Tam, Josie Bard, Devron R. Averett.).

In still further alternative aspects, alkylated sugar nucleosides (*e.g.*, 2'-methyl-, 3'-methyl-, 4'-methyl- and 5'-methyl-nucleosides) and 2',3'-dideoxy nucleosides may be synthesized as discussed in J. Med. Chem. 43 (2000) 3704-3713 (Synthesis and cytotoxicity of 4-amino-5-oxopyrido[2,3-d]pyrimidine nucleosides. Jean-Luc Girardet, Esmir Gunic, Cathey Esler, Dariusz Cieslak, Zbigniew Pietrzkowski, Guangyi Wang).

In yet further alternative aspects, dioxolane nucleoside compounds may be prepared as described in J. Med. Chem. 42 (1999) 2212-2217 (Structure activity relationships of L-dioxolane uracil nucleosides as anti-Epstein Barr virus agents. Ju-Sheng Lin, Toshihiko Kira, Elizabeth Gullen, Yongseok Choi, Fucheng Qu, Chung K. Chu, Yung-Chi Cheng). 2'-Fluoro- and 3'-fluoro nucleoside compounds may be synthesized as discussed in Organic Letters **3** (2001) 4177-4180 (Synthesis and potent anti-HIV activity of L-3'-fluoro-2',3'-unsaturated cytidine. Giuseppe Gumina, Raymond F. Shinazi and Chung K. Chu) and in J. Org. Chem. **66** (2001) 7469-7477 (Synthesis of 9-(2,3-dideoxy-2-fluoro-beta-D-threo-pentofuranosyl)adenine (FddA) via a purine 3'-deoxynucleoside. Satochi Takamatsu, T. Maruyama, S Katayama, N. Hirose, M. Naito, K. Izawa) and J. Med. Chem. **43** (2000) 2473-2478 (Synthesis of fluorosugar analogues of 2,5,6-trichloro-1-(beta-D-ribofuranosyl)benzimidazole as antivirals with potentially increased glycosidic bond stability. K. S. Gudmundsson, G.A. Freeman, J.C. Drach, L.B. Townsend).

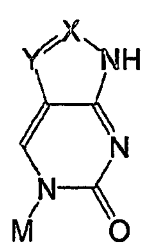
3'-azido and 3'-amino-nucleoside compounds may be synthesized as discussed in J. Org. Chem. **66** (2001) 8204-8210 (Syntheses of puromycin from adenosine and 7-deazapuromycin from tubercidin, and biological comparisons of the 7-aza/deaza pair. M.J. Robins, R.W. Miles, M.C. Samano, R.L. Kaspar). Azido nucleosides may then be converted to the corresponding nitro and/or amino groups via oxidation or reduction, and amino groups in such nucleosides may further act as nucleophiles for further derivatization.

3'-Deoxy-nucleoside compounds may be synthesized as discussed in Tetrahedron Letters 42 (2001) 561-563 (Efficient synthesis of protected 3'-deoxyadenosine and 3'deoxyguanosine from adenosine and guanosine. Z. Cui, B. Zhang). Synthesis for still further known sugar analogs may be found in "Modern Methods in Carbohydrate Synthesis" by Shaheer H. Khan (Gordon & Breach Science Pub; ISBN: 3718659212), in U.S. Pat Nos. 4,880,782 and 3,817,982, in WO88/00050, or in EP199,451 (*supra*).

With respect to the coupling of alternative sugars to the heterocyclic bases, it is generally contemplated that all, or almost all of the alternative sugars may be coupled to the heterocyclic base following a protocol as outlined in Schemes 3 or 11 above (and also as exemplified in the experimental section below). However, covalent coupling of the heterocyclic bases may also occur at a carbon atom other than the C1'-atom (*e.g.*, at the C2'-, C3'-, or C5'-atom where appropriate), and may be in either alpha or beta orientation.

**Use of Contemplated Compounds**

The inventors surprisingly discovered that contemplated compounds have significant inhibitory effect on various polymerases, and especially viral polymerases. Therefore, the inventors generally contemplate a method of inhibiting a polymerase in which in one step the viral polymerase is presented with a compound according to Formula 1 at a concentration effective to inhibit the viral polymerase



*Formula 1*

wherein -X=Y- is -N=N-, -CH=N-, -N=CZ- or -CH=CZ-, wherein Z is H, halogen, or alkyl, and wherein M is a sugar or sugar analog; and wherein the compound has a D-configuration or an L-configuration. With respect to suitable sugars for antiviral use of compounds according to Formula 1, the same considerations as discussed for contemplated sugars above apply.

The term "inhibiting a [viral] polymerase" as used herein refers to any reduction in catalytic activity, affinity to a substrate or one or more co-substrates, polymerization fidelity, and/or rate of polymerization of a [viral] polymerase. Thus, inhibition of a polymerase includes competitive, non-competitive, and/or allosteric inhibition, as well as inhibition caused at least in part by conformational changes in the polymerase, and suicidal inhibition. Moreover, the term "polymerase" as used herein is meant to encompass single polypeptide enzymes as well as polypeptide complexes with several subunits, and further includes DNA-dependent DNA polymerases, DNA-dependent RNA polymerases, RNA-dependent DNA polymerases, and RNA-dependent RNA polymerases.

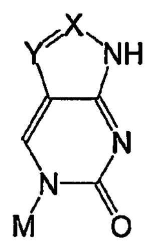
Therefore, it is contemplated that suitable polymerases include viral, bacterial, and eukaryotic polymerases. For example, suitable viral polymerases include those from a virus belonging to the family of *Flaviviridae*, and especially the HCV RNA-dependent RNA polymerase (NS5B). However, alternative viral polymerases include those belonging to the RSV virus, HBV virus, HIV virus, and the Influenza virus. Exemplary bacterial polymerases include those belonging to the genera *Bacillus, Streptococcus, Staphylococcus, Thermophilus*, and *Escherichia*. Contemplated eukaryotic polymerases particularly include those found in neoplastic tissue from a mammal, and especially from human.

Consequently, depending on the type of polymerase and environments in which the polymerase is located, the step of presenting the polymerase with contemplated compounds may vary considerably. For example, where the polymerase is a reverse transcriptase or a thermostable DNA-dependent DNA polymerase for *in vitro* protocols (*e.g.*, rtPCR, or qPCR), the step of presenting may include pipetting a suitable quantity of a previously prepared stock solution of contemplated compounds to a reaction mixture containing the polymerase. On the other hand, where the polymerase is in a cell of a cell culture, the step of presenting may include incubating the cells in a medium that contains contemplated compounds. Consequently, in such applications, the step of presenting may require uptake of the contemplated compounds into the cell.

Similarly, where the polymerase is in a cell of an animal (*e.g.*, human), the step of presenting may include administration of contemplated compounds using a suitable formulation, route, dosage, and protocol (see below). Consequently, where contemplated compounds are administered to an organism, the step of presenting may require administration, uptake of the contemplated compounds into the cell, and optionally

conversion of the compound into an active metabolite from contemplated compounds, or optionally conversion of a prodrug form of contemplated compounds into contemplated compounds.

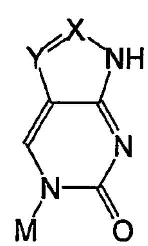
In still further preferred aspects of the inventive subject matter, the inhibitory effect on polymerases may be employed to treat various diseases, and especially contemplated diseases include viral infections and neoplastic diseases. Therefore, the inventors contemplate a method of treating a viral infection in a patient in which a compound according to Formula 1 is administered to the patient at a dosage effective to reduce viral propagation in the patient



*Formula 1*

wherein -X=Y- is -N=N-, -CH=N-, -N=CZ- or -CH=CZ-, wherein Z is H, halogen, or alkyl, and wherein M is a sugar or sugar analog; and wherein the compound has a D-configuration or an L-configuration. With respect to the sugar, and contemplated viruses the same considerations as discussed immediately above apply.

Consequently, the inventors contemplate a pharmaceutical composition comprising a compound according to Formula 1



*Formula 1*

wherein -X=Y- is -N=N-, -CH=N-, -N=CZ- or -CH=CZ-, wherein Z is H, halogen, or alkyl, and wherein M is a sugar or sugar analog; wherein the compound has a D-

configuration or an L-configuration; and wherein the compound is present at a concentration effective to inhibit propagation of a virus in a patient to which the composition is administered

Especially preferred compounds in contemplated pharmaceutical compositions further comprise a phosphate, thiophosphate, phosphonate, or phosphoamidate group that is covalently coupled to the sugar or sugar analog (and most preferably at the C5'-position where the sugar is in a furanose form). As already discussed above in the section "Contemplated Compounds", particularly preferred compounds in contemplated pharmaceutical compositions will further include a moiety that increases selectivity of the compound to a target cell (*e.g.*, neoplastic cell or cell of a particular cell type such as a hepatocyte), wherein at least part of the moiety is removed from the compound in the target cell (*e.g.*, in an enzymatic oxido-reduction, hydrolysis, or other enzymatic or non-enzymatic reaction). Consequently, preferred moieties include those in which the moiety forms an ester or cyclic diester with a phosphate, thiophosphate, phosphonate, or phosphoamidate group.

Therefore, pharmaceutical compositions according to the inventive subject matter generally include contemplated compounds of Formula 1 (above) or a pharmaceutically acceptable salt thereof, and may further comprise a pharmaceutically acceptable carrier and optionally other therapeutic ingredients. Especially preferred compositions include compositions suitable for oral, rectal, topical, parenteral (including subcutaneous, intramuscular, and intravenous), ocular (ophthalmic), pulmonary (nasal or buccal inhalation), or nasal administration, although the most suitable route in any given case will depend on the nature and severity of the conditions being treated and on the nature of the active ingredient.

Contemplated pharmaceutical compositions may be presented in unit dosage form and prepared by any of the methods well known in the art of pharmacology. Therefore, contemplated compounds may be combined as the active ingredient in admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, *e.g.*, oral or parenteral (including intravenous). In preparing the compositions for oral dosage form, any of the usual pharmaceutical media may be employed, for example, water, glycols, oils, alcohols, flavoring agents, preservatives,

coloring agents, etc. (for an oral liquid preparation, including suspensions, elixirs, and solutions). Alternatively, suitable carriers for oral solid preparations include starch, sugar, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, etc. (e.g., for powders, hard and soft capsules, or tablets).

Tablets and capsules represent the most advantageous oral dosage unit form in which solid pharmaceutical carriers may be employed. If desired, tablets may be coated by standard aqueous or nonaqueous techniques. Such compositions and preparations will typically contain at least 0.1 percent of contemplated compound. The percentage of contemplated compound in these compositions may, of course, be varied and may vary between about 0.5 percent to about 95 percent of the weight of the unit. The amount of contemplated compound in such therapeutically useful compositions is such that an effective dosage will be obtained.

The tablets, pills, capsules, etc. may also contain a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin. When a dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier such as a fatty oil. Various other materials may further be present as coatings or to modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both. A syrup or elixir may contain, in addition to the active ingredient, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and a flavoring such as cherry or orange flavor.

Alternatively, contemplated compounds may also be administered parenterally, and solutions and/or suspensions of these active compounds can be prepared in water suitably mixed with a surfactant such as hydroxy-propylcellulose. Alternatively, dispersions may be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example,

water, ethanol, polyol (e. g. glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

Thus, it should be recognized that various routes of administration may be employed for providing a mammal, especially a human with an effective dosage of contemplated compounds. For example, oral, rectal, topical, parenteral, ocular, pulmonary, nasal, and the like may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, and the like. However, it is generally preferred that contemplated compounds are administered orally.

For oral administration to humans, the dosage range is 0.01 to 1000 mg/kg body weight in divided doses. In one embodiment the dosage range is 0.1 to 100 mg/kg body weight in divided doses. In another embodiment the dosage range is 0.5 to 20 mg/kg body weight in divided doses. For oral administration, the compositions are preferably provided in the form of tablets or capsules containing 1.0 to 1000 milligrams of the active ingredient. However, the effective dosage of active ingredient employed may vary depending on the particular compound employed, the mode of administration, the condition being treated and the severity of the condition being treated. Such dosage may be ascertained readily by a person skilled in the art. This dosage regimen may be adjusted to provide the optimal therapeutic response.

Where contemplated compounds are employed in the treatment of an HCV infection, it should be recognized that contemplated compounds may also be administered in combination with one or more agents useful for treating HCV infection. For example, suitable agents active against HCV especially include Ribavirin, Levovirin, Viramidine (in D- or L-configuration), Thymosin alpha-1, interferon-alpha, pegylated interferon-alpha (*e.g.*, peginterferon-alpha), and all reasonable combinations thereof.

Coadministration of contemplated compounds with one or more agents useful for treating HCV infection may be separated at different times during the course of therapy or concurrent in divided or single combination forms. Thus, coadministration expressly includes all regimes of simultaneous or alternating treatment. It should be understood that the scope of combinations of the compounds of this invention with other agents useful for treating HCV infection includes in principle any combination with any pharmaceutical composition for treating HCV infection. When a compound of the present invention or a

pharmaceutically acceptable salt thereof is used in combination with a second therapeutic agent active against HCV, the dose of each compound may be either the same as or different from the dose when the compound is used alone.

Furthermore, the compounds of the present invention may also be administered in combination with an agent that is an inhibitor of HCV NS3 serine protease. Both substrate and non-substrate based inhibitors of HCV NS3 protease inhibitors are disclosed in WO 98/17679, WO 98/22496, WO 98/46630, WO 99/07733, WO 99/07734, WO 99/38888, WO 99/50230, WO 99/64442, WO 00/09543, WO 00/59929, WO 01/74768, WO 01/81325, and GB- 2337262.

## Examples

The following examples are provided to further illustrate exemplary synthesis and use of contemplated compounds. However, it should be appreciated that numerous alternative protocols and uses are also suitable.

*Scheme 1*

a.) 2-amino-7*H*-pyrrolo[2,3-*d*]pyrimidine (**2**). To a suspension of 2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**1**) (1.0 g, 6.0 mmol) in MeOH (50 mL) and aqueous ammonium hydroxide (1 mL) was added 10% Pd-C (500 mg). The reaction mixture was stirred under atmospheric pressure for 17 h and the catalyst was filtered through a pad of Celite. The filtrate was concentrated and chromatographed with silica gel ($CH_2Cl_2$:MeOH = 90:10) to yield 717 mg of the target compound (90%). $^1$H NMR (DMSO-*d6*): δ 11.4 (bs, 1H), 8.50 (s, 1 H), 7.11 (dd, $J$ = 3.6, 0.6 Hz, 1H), 6.49 (bs, 2H), 6.32 (dd, $J$ = 3.6, 0.9 Hz, 1 H).

b.) 2-amino-7-benzyloxycarbonyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**3**). To a solution of the compound **2** (717 mg, 5.4 mmol), triethylamine (0.89 mL, 6.42 mmol), and DMAP (10 mg) in DMF (10 mL) at 0 °C was added benzylchloroformate (0.15 mL, 29 mmol). After 30 min, the mixture was warmed to room temperature and stirred for 24 hrs. The mixture was diluted with $CH_2Cl_2$ (100 mL) and the organic solution was washed with brine (100 mL), dried with $Na_2SO_4$, and concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 97:3) yielded 746 mg of the target compound (52%). $^1$H NMR ($CDCl_3$):

δ 8.51 (s, 1 H), 7.51-7.34 (m, 6H), 6.41 (dd, $J$ = 4.2, 0.6 Hz, 1H), 5.45 (s, 2H), 5.32 (bs, 2H).

c.) 2-hydroxy-7-benzyloxycarbonyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (**4**). To a suspension of the compound **3** (921 mg, 3.4 mmol) in $H_2O$ (70 mL) and acetic acid (9 mL) at 65 °C was added sodium nitrite (2.37g, 34 mmol) in portions. After 40 min, the reaction was cooled down to room temperature and extracted with $CHCl_3$ (5 x 100 mL). The combined organic solution was washed with brine (200 mL), dried with $Na_2SO_4$, and concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 95:5) yielded 472 mg of the target compound (51%). $^1$H NMR ($CDCl_3$): δ 8.25 (s, 1 H), 7.53-7.37 (m, 6H), 6.38 (dd, $J$ = 4.2, 0.6 Hz, 1H), 5.50 (s, 2H).

d.) 3-(2',3',5'-tri-*O*-benzoyl –β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-one (**6a**). To a solution of the compound **4** (143 mg, 0.53 mmol) in $CH_3CN$ (10 mL) was added *N*,*O*-bis(trimethylsilyl)acetamide (0.26 ml, 1.1 mmol). The reaction mixture was stirred at 80 °C for 20 min and cooled down to room temperature. To the mixture were added 2,3,5-tri-*O*-benzoyl-1-*O*-acetyl-β-D-ribofuranose (**5a**) (320 mg, 0.63 mmol) and $SnCl_4$ (0.85 mL, 0.84 mmol). The reaction mixture was stirred at 90 °C for 2 h, cooled down to room temperature and diluted with $CH_2Cl_2$ (100 mL). The organic solution was washed with aqueous $NaHCO_3$ solution (100 mL) and brine (100 mL), dried with $Na_2SO_4$, and concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 95:5) yielded 138 mg of the target compound (45%). $^1$H NMR ($CDCl_3$): δ 6.60 (d, $J$ = 3.0 Hz, 1H, H-1').

e.) 3-(2',3',5'-tri-*O*-benzoyl-2'-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-one (**6b**). To a solution of the compound **4** (143 mg, 0.53 mmol) in 1,2-dichlorethane (10 mL) was added *N*,*O*-bis(trimethylsilyl)acetamide (0.26 ml, 1.1 mmol). After 15 min, 2,3,5-tri-*O*-benzolyl-1-*O*-acetyl-2-methyl-β-D-ribofuranose (**5b**) (320 mg, 0.63 mmol) and trimethylsilyl trifluoromethanesulfonate (0.28 mL, 1.6 mmol) were added to the mixture. The reaction mixture was stirred at room temperature for 3 days and diluted with $CH_2Cl_2$ (100 mL). The organic solution was washed with aqueous $NaHCO_3$ solution (20 mL) and brine (20 mL), dried with $Na_2SO_4$, and concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 99:1) yielded 138 mg of the target compound (45%). $^1$H NMR ($CDCl_3$): δ 6.96 (s, 1H, H-1').

f.) 3-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-one (**7a**). A solution of the compound **6a** (40 mg, 0.069 mmol) in methanolic ammonia (20 mL) was stirred at room temperature in a sealed bomb for 15 h. The bomb was cooled to 0 °C before opening. The reaction mixture was stirred at RT for 1 h and then concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 80:20) yielded 18 mg of the target compound (98%). $^1H$ NMR ($CD_3OD$): δ 6.02 (d, *J* = 1.8 Hz, 1H, H-1').

g.) 3-(2'-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-one (**7b**). A solution of the compound **6b** (120 mg, 0.20 mmol) in methanolic ammonia (30 mL) was stirred at room temperature in a sealed bomb for 15 h. The bomb was cooled to 0 °C before opening. The reaction mixture was stirred at RT for 1 h and then concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 80:20) yielded 53 mg of the target compound (95%). $^1H$ NMR ($CD_3OD$): δ 6.27 (s, 1H, H-1').

*Scheme 2*

a.) 3-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-one-5'-triphosphate (**8a**). A mixture of the compound **7a** (21 mg, 0.079 mmol), anhydrous trimethylphosphonate (1 mL) and molecular sieves were gently stirred at room temperature overnight. The mixture was cooled to 0 °C and phosphorous oxychloride (19 μL, 0.20 mmol) was added. The reaction was stirred at 0 °C for 2 h. To the mixture were added tributyl amine (96 μL, 0.40 mmol), anhydrous acetonitrile (300 μL) and tibutylammonium pyrophosphate (228 mg, 0.50 mmol). After stirring at 0 °C for 3 h, the reaction mixture was poured into a cooled solution of triethylammnium bicaronate buffer (0.5 M, pH 8.0, 5 mL) and purified by HPLC (YMC ODS-AQ column, 0-10% $CH_3CN$ in 100 mM triethylammonium acetate) to yield 8 mg of the target compound (20%).

b.) 3-(2'-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-one-5'-triphosphate (**8b**). A mixture of the compound **7a** (24 mg, 0.085 mmol), anhydrous trimethylphosphonate (1 mL) and molecular sieves were gently stirred at room temperature overnight. The mixture was cooled to 0 °C and phosphorous oxychloride (19 μL, 0.20 mmol) was added. The reaction was stirred at 0 °C for 2 h. To the mixture were added tributyl amine (96 μL, 0.40 mmol), anhydrous acetonitrile (300 μL) and tibutylammonium pyrophosphate (228 mg, 0.50 mmol). After stirring at 0 °C for 3 h, the reaction mixture was

poured into a cooled solution of triethylammnium bicaronate buffer (0.5 M, pH 8.0, 5 mL) and purified by HPLC (YMC ODS-AQ column, 0-10% $CH_3CN$ in 100 mM triethylammonium acetate) to yield 9 mg of the target compound (20%). $^1$H NMR ($CD_3OD$): δ 6.00 (d, $J$ = 1.5 Hz, 1H, H-1').

c.) 3-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-one-5'-[bis-(*S*-pivaloyl-2-thioethyl)phosphate] (**9a**). To a mixture of the compound **8a** (21 mg, 0.079 mmol), bis(*S*-pivaloyl-2-thioethyl)-*N,N*-diisopropylphosphoramidite (70 μL, 0.16 mmol) in DMF (1.5 mL) was added $^1$H-tetrazole (17 mg, 0.24 mmol). After stirring at room temperature for 45 min, the mixture was cooled to –40 °C and *tert*- butylhydroperoxide (43 μL, 5M in decane, 0.21 mmol) was added to the mixture. The reaction was slowly warmed to room temperature and stirred for 2 h. The mixture was quenched by the addition of 10% solution of sodium bisulfite (1 mL) at 0 °C and the resulting mixture was extracted with $CHCl_3$ (2 x 5 mL). The organic solution was dried with $Na_2SO_4$, and concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 95:5) yielded 30 mg of the target compound (60%). $^1$H NMR ($CD_3OD$): δ 6.00 (d, $J$ = 1.5 Hz, 1H, H-1').

d.) 3-(2'-*C*-methyl-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-one-5'-[bis-(*S*-pivaloyl-2-thioethyl)phosphate] (**9b**). To a mixture of the compound **8b** (45 mg, 0.16 mmol), bis(*S*-pivaloyl-2-thioethyl)-*N,N*-diisopropylphosphoramidite (140 μL, 0.32 mmol) in DMF (3 mL) was added $^1$H-tetrazole (34 mg, 0.48 mmol). After stirring at room temperature for 45 min, the mixture was cooled to –40 °C and *tert*- butylhydroperoxide (80 μL, 5M in decane, 0.42 mmol) was added to the mixture. The reaction was slowly warmed to room temperature and stirred for 2 h. The mixture was quenched by the addition of 10% solution of sodium bisulfite (1 mL) at 0 °C and the resulting mixture was extracted with $CHCl_3$ (2 x 10 mL). The organic solution was dried with $Na_2SO_4$, and concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 95:5) yielded 58 mg of the target compound (53%). $^1$H NMR ($CD_3OD$): δ 6.29 (s, 1H, H-1').

*Scheme 3*

a.) 3-(2',3',5'-tri-*O*-acetyl –β-L-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-one (**6a-L**). A solution of the compound 4 (143 mg, 0.53 mmol) in $CH_3CN$ (10 mL) was added and *N,O*-bis(trimethylsilyl)acetamide (0.26 ml, 1.1 mmol). The reaction mixture was stirred

at 80 °C for 20 min and cooled down to room temperature. To the mixture were added tetra-*O*-acetyl-β-L-ribofuranose (**5a-L**) (201 mg, 0.63 mmol) and $SnCl_4$ (0.85 mL, 0.84 mmol). The reaction mixture was stirred at 90 °C for 2 h, cooled down to room temperature and diluted with $CH_2Cl_2$ (100 mL). The organic solution was washed with aqueous $NaHCO_3$ solution (100 mL) and brine (100 mL), dried with $Na_2SO_4$, and concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 95:5) yielded 132 mg of the target compound (43%).

b.) 3-(β-L-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-one (**7a-L**). A solution of the compound **6a-L** (132 mg, 0.23 mmol) in methanolic ammonia (25 mL) was stirred at room temperature in a sealed bomb for 15 h. The bomb was cooled to 0 °C before opening. The reaction mixture was stirred at room temperature for 1 h and then concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 80:20) yielded 58 mg of the target compound (95%).

c.) 3-[(3,5-*O*-tetraisopropyl-1,3-disiloxanediyl)-β-L-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-one (**10a-L**). TIPDS-Cl (1.3 mL. 4.1 mmol) was added to a stirred solution of the compound **7a-L** (909 mg, 3.4 mmol) in pyridine (10 mL) at 0 °C. The reaction mixture was stirred at room temperature for 20 h, then cooled to 0 °C, quenched with water (0.5 mL), and concentrated to dryness. The residue was diluted with $CH_2Cl_2$ (100 mL) and the organic solution was washed with aqueous $NaHCO_3$ solution (100 mL) and brine (100 mL), dried with $Na_2SO_4$, and concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 95:5) yielded 1.59 g of the target compound (92%).

d.) 3-(2-deoxy-β-L-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-one (**12a-L**). A solution of the compound **10a-L** (612 mg, 1.2 mmol), DMAP (0.30 g, 2.4 mmol), and phenyl chlorothionoformate (0.19 mL, 1.3 mmol) in acetonitrile (10 mL) was stirred at room temperature for 2 h and then concentrated to dryness. The residue was diluted with $CH_2Cl_2$ (100 mL) and the organic solution was washed with aqueous $NaHCO_3$ solution (100 mL) and brine (100 mL), dried with $Na_2SO_4$, and concentrated to dryness. The residue **11a-L** was dissolved in toluene (10 mL), and tris(trimethylsilyl)silane (0.56 mL, 1.8 mmol) and 1,1'-azobis(cyclohexanecarbonitrile) (74 mg, 0.30 mmol) were added. The reaction mixture was heated at 80 °C for 2 h and at 105 °C for 15 h. The solvent was evaporated and the residue was dissolved in THF (5 mL). TBAF (1.0 M, 2.5 mL) was added and the reaction

mixture was stirred at room temperature for 2 h. The solvent was evaporated and the residue was chromatographed ($CH_2Cl_2$:MeOH = 90:10) to yield 214 mg of the target compound (71% from **10a-L**).

*Scheme 4*

Formation of the triphosphate and the bis-pivaloyl ester prodrug form for the compounds of Scheme 3 followed substantially the same conditions as those described in Scheme 2 above.

*Scheme 5*

a.) 3-(5'-*O*-*tert*-butyldiphenylsilyl-2',3'-di-*O*-methanesulfonyl–β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-one (**15a**). To a solution of the compound **7a** (681 mg, 2.6 mmol) and DMAP (370 mg, 3.0 mmol) in pyridine (10 mL) at 0 °C was added TBDPS-Cl (0.80 mL, 3.0 mmol). The mixture was stirred at room temperature for 20 h and concentrated to dryness. The residue was diluted with EtOAc (100 mL) and the organic solution was washed with brine (100 mL), dried with $Na_2SO_4$, and concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 95:5) yielded 5'-TBDPS protected compound. To a solution of the compound obtained above in pyridine (10 mL) was added Ms-Cl (0.37 mL, 4.7 mmol) at 0 °C. The mixture was stirred at room temperature for 3 h, quenched with MeOH (0.2 mL) and concentrated to dryness. The residue was diluted with EtOAc (100 mL) and the organic solution was washed with brine (100 mL), dried with $Na_2SO_4$, and concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 93:7) yielded 1.36 g of the target compound (79%).

b.) 3-(5'-*O*-*tert*-butyldiphenylsilyl-2',3'-didehydro-2'.3'-dideoxy–β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-one (**16a**). To a Te powder (200 mesh, 113 mg, 0.89 mmol) in dry round bottom flask equipped with reflux condenser under argon was added $Et_3BHLi$ (2.0 mL, 2.0 mmol, 1 M in THF). The mixture was stirred for 6 h and a solution of the compound **15a** (245 mg, 0.37 mmol) in THF (2 mL) was added to the mixture. After stirring at room temperature for 20 h, the mixture was concentrated. Silica gel chromatography ($CH_2Cl_2$:MeOH = 98:2) yielded 136 mg of the target compound (78%).

c.) 3-(2'.3'-dideoxy–β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-one (**17a**). A mixture of the compound **16a** (136 mg, 0.29 mmol) and Pd-C (50 mg, 10%) in MeOH

(10 mL) was stirred under $H_2$ for 16 h. The mixture was filtered through Celite and concentrated to dryness. To the residue was added TBAF (0.35 mL, 0.35 mmol, 1 M in THF). After stirring at room temperature for 16 h, the reaction was concentrated. Silica gel chromatography ($CH_2Cl_2$:MeOH = 90:10) yielded 48 mg of the target compound (70%).

*Scheme 6*

Formation of the triphosphate and the bis-pivaloyl ester prodrug form for the compounds of Scheme 5 followed substantially the same conditions as those described in Scheme 2 above.

*Scheme 7*

a.) 5-bromo-1-(2'-*C*-methyl-β-D-ribofuranosyl)-*1H*-pyrimidin-2,4-dione (**21b**). To a solution of 1-(2'-methyl-2',3',5'-tri-*O*-benzolyl-β-D-ribofuranosyl)-$^1H$-pyrimidine-2,4-dione (**20b**) (4.0 g, 7.0 mmol) in acetonitrile (60 mL) was added ammonium cerium(IV) nitrate (1.92 g, 3.5 mmol) and lithium bromide (695 mg, 8.0 mmol). After stirring at 90 °C for 16 h, the mixture was cooled down to room temperature and diluted with $CH_2Cl_2$ (200 mL). The organic solution was washed with brine (100 mL), dried with $Na_2SO_4$, and concentrated to dryness. Silica gel chromatography (EtOAc:Hexanes = 50:50) yielded 5-bromo-1-(2'-methyl-2',3',5'-tri-*O*-benzolyl-β-D-ribofuranosyl)-$^1H$-pyrimidine-2,4-dione. To the compound obtained was added methanolic ammonia (100 mL) in a sealed bomb. After stirring at room temperature for 16 h, the reaction was concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 80:20) yielded 1.20 g of the target compound (51%).

b.) 5-bromo-1-(2'-*C*-methyl-β-D-ribofuranosyl)-*1H*-pyrimidine-2,4-dione (**22b**). To a solution of **21b** (1.20 g, 3.6 mmol) in pyridine (30 mL) was added TMSCl (3.60 mL, 29 mmol) and the resulting solution was stirred at room temperature for 1 h. To the mixture was added $POCl_3$ (0.66 ml, 7.2 mmol) and the resulting solution was stirred at room temperature for 4 h. The reaction was cooled to 0 °C and quenched by the addition of water (10 mL). To the mixture was added a solution of concentrated ammonium hydroxide (90 mL) and the resulting solution was heated at 50 °C for 16 h. The solution was concentrated to dryness and chromatographed with silica gel ($CH_2Cl_2$:MeOH = 80:20) to yield 430 mg of the target compound (36%).

c.) 5-amino-cytidine (**23a**). A solution of 5-bromocytidine (**22a**) (500 mg, Sigma, 1.6 mmol) in liquid ammonia (20 mL) was stirred at 70 °C in a sealed bomb for 16 h. The bomb was cooled to –78 °C before opening and the liquid ammonia was evaporated to yield the target compound, which was used for the next reaction without purification.

d.) 5-amino-1-(2'-*C*-methyl-β-D-ribofuranosyl)-*1H*-pyrimidin-2,4-dione (**23b**). A solution of compound **22b** (420 mg, 1.3 mmol) in liquid ammonia (20 mL) was stirred at 70 °C in a sealed bomb for 16 h. The bomb was cooled to –78 °C before opening and the liquid ammonia was evaporated to yield the target compound, which was used for the next reaction without purification.

e.) 1-(β-D-ribofuranosyl)-2-oxypurine (**24a**). The compound **23a** was suspended in diethoxymethyl acetate (10 mL) and the suspension was heated to reflux for 2 h. The mixture was diluted with $CH_2Cl_2$ (100 mL), washed with brine (100 mL), dried with $Na_2SO_4$, and concentrated to dryness. The resulting brown oil was dissolved in 5% solution of trifluoroacetic acid in a mixture of $CH_2Cl_2$ and MeOH (75:25, 10 mL) at 0 °C. After 3 h at 0 °C, the reaction was concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 80:20) yielded 77 mg of the target compound (18% from **22a**). $^1$H NMR ($CD_3OD$): δ 5.87 (d, $J$ = 3.9 Hz, 1H, H-1').

f.) 1-(2'-*C*-methyl-β-D-ribofuranosyl)-2-oxypurine (**24b**). The compound **23b** was suspended in diethoxymethyl acetate (10 mL) and the suspension was heated to reflux for 2 h. The mixture was diluted with $CH_2Cl_2$ (100 mL), washed with brine (100 mL), dried with $Na_2SO_4$, and concentrated to dryness. The resulting brown oil was dissolved in 5% solution of trifluoroacetic acid in a mixture of $CH_2Cl_2$ and MeOH (75:25, 10 mL) at 0 °C. After 3 h at 0 °C, the reaction was concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 80:20) yielded 84 mg of the target compound (23% from **22b**).

*Scheme 8*

Formation of the triphosphate and the bis-pivaloyl ester prodrug form for the compounds of Scheme 7 followed substantially the same conditions as those described in Scheme 2 above.

*Scheme 9*

a.) 6-(β-D-ribofuranosyl)-1,6-dihydro-[1,2,3]triazolo[4,5-*d*]-pyrimidin-5-one (**27a**). To a solution of the compound **23a** (321 mg, 1.2 mmol) in 1 mL of 2N HCl solution at 0 °C was added sodium nitrite (99 mg, 1.4 mmol) slowly in portions. After 1 h, a white precipitate was filtered to yield 171 mg of the target compound (51%).

b.) 6-(2'-*C*-methyl-β-D-ribofuranosyl)-1,6-dihydro-[1,2,3]triazolo[4,5-*d*]-pyrimidin-5-one (**27b**). To a solution of the compound **23b** (120 mg, 0.44 mmol) in 1 mL of 2N HCl solution at 0 °C was added sodium nitrite (36 mg, 0.53 mmol) slowly in portions. After 1 h, a white precipitate was filtered to yield 29 mg of the target compound (23%).

*Scheme 10*

Formation of the triphosphate and the bis-pivaloyl ester prodrug form for the compounds of Scheme 9 followed substantially the same conditions as those described in Scheme 2 above.

*Scheme 11*

a.) 6-amino-1*H*-pyrazolo[3,4-*d*]pyrimidine (**31**). To a suspension of 6-amino-4-chloro-*1H*-pyrazolo[3,4-*d*]pyrimidine (**30**) (2.3 g, 14 mmol) in MeOH (200 mL) and aqueous ammonium hydroxide (4 mL) was added 10% Pd-C (500 mg). The reaction mixture was stirred under atmospheric pressure for 16 h and the catalyst was filtered through a pad of Celite. The filtrate was concentrated and chromatographed with silica gel ($CH_2Cl_2$:MeOH = 90:10) to yield 1.1 g of the target compound (60%).

b.) 6-amino-1-benzoyloxycarbonyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**32**). To a solution of the compound **31** (0.41 g, 3.0 mmol), triethylamine (0.51 mL, 3.7 mmol), and DMAP (10 mg) in DMF (15 mL) at 0 °C was added benzylchloroformate (0.55 mL, 3.7 mmol). After 10 min, the mixture was warmed to room temperature and stirred for 16 h. The mixture was diluted with $CH_2Cl_2$ (100 mL) and the organic solution was washed with aqueous $NaHCO_3$ solution (50 mL) and brine (50 mL), dried with $Na_2SO_4$, and concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 95:5) yielded 0.41 g of the target compound (50%).

c.) 6-hydroxy-1-benzoyloxycarbonyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**33**). To a suspension of the compound **32** (0.46 g, 1.7 mmol) in $H_2O$ (35 mL) and acetic acid (4.5 mL) at 60 °C was added sodium nitrite (1.2 g, 17 mmol) in small portion. After 30 min, the reaction was cooled down to room temperature and extracted with $CHCl_3$ (5 x 50 mL). The combined organic solution was washed with brine (100 mL), dried with $Na_2SO_4$, and concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 95:5) yielded 0.22 g of the target compound (48%).

d.) 5-(2',3',5'-tri-*O*-benzolyl –β-D-ribofuranosyl)- 1*H*-pyrazolo[3,4-*d*]pyrimidin-6-one (**34a**). To a solution of the compound **33** (0.22 g, 0.81 mmol) in $CH_3CN$ (10 mL) was added *N*,*O*-bis(trimethylsilyl)acetamide (0.39 ml, 1.7 mmol). The reaction mixture was stirred at 80 °C for 20 min and cooled down to room temperature. To the mixture were added 2,3,5-tri-*O*-benzoly-1-*O*-acetyl-β-D-ribofuranose (**5a**) (0.48 g, 0.95 mmol) and $SnCl_4$ (1.28 mL, 1.3 mmol). The reaction mixture was stirred at 90 °C for 2 h, cooled down to room temperature and diluted with $CH_2Cl_2$ (100 mL). The organic solution was washed with aqueous $NaHCO_3$ solution (100 mL) and brine (100 mL), dried with $Na_2SO_4$, and concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 95:5) yielded 0.21 g of the target compound (45%).

e.) 5-(2',3',5'-tri-*O*-benzolyl-2'-*C*-methyl-β-D-ribofuranosyl)- 1*H*-pyrazolo[3,4-*d*]pyrimidin-6-one (**34b**). To a solution of the compound **33** (70 mg, 0.26 mmol) in 1,2-dichlorethane (5 mL) was added *N*,*O*-bis(trimethylsilyl)acetamide (0.13 ml, 0.54 mmol). After 15 min, 2,3,5-tri-*O*-benzolyl-1-*O*-acetyl-2-methyl-β-D-ribofuranose (**5b**) (0.16 g, 0.31 mmol) and trimethylsilyl trifluoromethanesulfonate (0.14 mL, 0.78 mmol) were added to the mixture. The reaction mixture was stirred at room temperature for 3 days and diluted with $CH_2Cl_2$ (50 mL). The organic solution was washed with aqueous $NaHCO_3$ solution (20 mL) and brine (20 mL), dried with $Na_2SO_4$, and concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 99:1) yielded 80 mg of the target compound (42%).

f.) 5-(β-D-ribofuranosyl)- 1*H*-pyrazolo[3,4-*d*]pyrimidin-6-one (**35a**). A solution of the compound **34a** (100 mg, 0.17 mmol) in methanolic ammonia (20 mL) was stirred at room temperature in a sealed bomb for 16 h. The bomb was cooled to 0 °C before opening. The reaction mixture was stirred at room temperature for 1 h and then concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 80:20) yielded 43 mg of the target compound (95%).

g.) 5-(2'-*C*-methyl-β-D-ribofuranosyl)- 1*H*-pyrazolo[3,4-*d*]pyrimidin-6-one (**35b**). A solution of the compound **34b** (80 mg, 0.11 mmol) in methanolic ammonia (10 mL) was stirred at room temperature in a sealed bomb for 16 h. The bomb was cooled to 0 °C before opening. The reaction mixture was stirred at room temperature for 1 h and then concentrated to dryness. Silica gel chromatography ($CH_2Cl_2$:MeOH = 80:20) yielded 25 mg of the target compound (82%).

*Scheme 12*

Formation of the triphosphate and the bis-pivaloyl ester prodrug form for the compounds of Scheme 11 followed substantially the same conditions as those described in Scheme 2 above.

*Biological Assays*

Numerous contemplated compounds were tested (data not shown), and exemplary data are provided for selected compounds.

*Phosphorylation of Compound 7a*

100 μM of 7a, or cytidine for control, were incubated with 100 nM of hUCK1 enzyme in the presence of 1 μM unlabeled ATP, 0.2 μCi of [$\gamma$-$^{32}$P]ATP, 5 mM $MgCl_2$ and 50 mM Tris-HCl, pH 7.5, in a total reaction volume of 50 μl. The reactions were incubated at 37°C for 1 hour, then stopped with 10 ul of 0.5 M EDTA. 2 μl of each reaction were spotted on a polyethyleneimine cellulose thin layer chromatography plate and the nucleosides were separated in 0.3 M potassium phosphate buffer, pH 7. The plate was exposed on a phosphor imager screen and bands were quantified using a Typhoon 8600 Imager (Amersham Pharmacia Biotech, Piscataway, NJ). The phosphorylation efficiency of 7a was 36% compared to that of cytidine.

*Incorporation of phosphorylated Compound 7a*

Incorporation of bicyclocytidine triphosphate by HCV NS5B through base-pairing with A into a product was examined by using an RNA template, 5'-AAAAAGGAGC-3' and a $^{33}$P labeled primer, pGpC. The reaction gave a trinucleotide product or longer

elongation products when additional CTP was present. Similarly, incorporation opposite to G was tested by using 5'-AAAAAAAGAU-3' and $^{33}$pApU.

*Assay Protocol*

A standard reaction was carried out in 10 μL containing 50 mM HEPES (pH 7.3), 10 mM DTT, 5 mM $MgCl_2$, 20 μM RNA template, 2.5 – 5 μM NS5B, 20 μM of 5'-$^{33}$P labeled primer, and 1 mM of elongating nucleotides as indicated. The reaction mixture was incubated at 30°C for 1 h, and then quenched by the addition of 10 μL loading buffer (90% formamide, 0.025% bromophenol blue and 0.025% xylene cyanol). The quenched reaction mixture was heated to 70°C for 2-5 min prior to loading 2-3 μL onto a denaturing 25% polyacrylamide-7 M urea-TBE gel. Electrophoresis was performed in 1X TBE at 70-90 watts. Gels were visualized and analyzed using a PhosphorImager.

The resulting products are shown in **Figures 1A and 1B** along with products from control experiments using UTP or CTP [Figure 1. Incorporation of compound 8a by NS5B using AAAAAGGAGC and $^{33}$pGpC (lane 1-5), and AAAAAAAGAU and $^{33}$pApU (lane 6-10). Each reaction contained 5 μM of NS5B, 20 μM of the primer, and 20 μM of the RNA template and 1 mM of a nucleotide as following: lane 1, none; lane 2, UTP; lane 3, UTP and CTP; lane 4, compound 8a; lane 5, compound 8a and CTP; lane 6, none; lane 7, CTP; lane 8, CTP and UTP; lane 9, compound 8a; and lane 10, compound 8a and UTP. The reaction products were resolved on a 25% polyacrylamide-7 M urea-TBE gel and were subjected to PhosphorImager analysis].

It appears that bicyclocytidine triphosphate was not incorporated into a product through base-pairing with A (lane 4), nor were there any elongation products (lane 5). However, the trinucleotide product was clearly seen when it was incorporated through base-pairing with G (lane 9). Intensity of the band is comparable of that of CTP incorporation. Interestingly, elongation products from both CTP and bicyclocytidine triphosphates were not clearly visible (lanes 8 and 10). This result indicates that bicyclocytosine exists as a cytosine analog rather than as a uracil analog under the given conditions of the experiment.

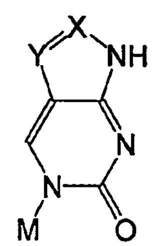
Thus, specific embodiments and applications of cytidine analogs and methods of use have been disclosed. It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the

inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the appended claims. Moreover, in interpreting both the specification and the claims, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms "comprises" and "comprising" should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

## CLAIMS

What is claimed is:

1. A compound having a structure according to Formula 1:



*Formula 1*

wherein -X=Y- is -N=N-, -CH=N-, -N=CZ- or -CH=CZ-, wherein Z is H, halogen, or alkyl, and wherein M is a sugar or sugar analog;

wherein the compound has a D-configuration or an L-configuration;

with the proviso that where M is a sugar with a ribofuranose ring having a heteroatom and substituents $R_1$ and $R_2$ on the C3'-atom, $R_3$ and $R_4$ on the C2'-atom, and $R_5$ on the C5'-atom, $R_1$, $R_2$, $R_3$, and $R_4$ together are not independently H, OH, F, $NH_2$, $N_3$, O-hydrocarbyl, or a reporter moiety, when the heteroatom is O, S, Se, SO, N-alkyl, or $CH_2$, and when $R_5$ is OH, SH, $NH_2$, monophosphate, diphosphate, triphosphate, thiophosphate, or boranophosphate; and

with the further proviso that M does not comprise a cyclopropenyl group, a morpholino group, or M is not a phosphonylmethoxyethyl.

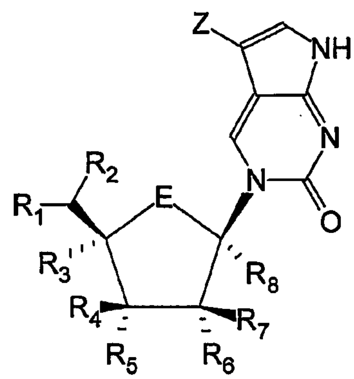
2. The compound of claim 1 further comprising a phosphate, thiophosphate, boranophosphate, phosphonate, or phosphoamidate group covalently coupled to the sugar or sugar analog.

3. The compound of claim 2 further comprising a moiety that increases selectivity of the compound to a target cell, and wherein at least part of the moiety is removed from the compound in the target cell.

4. The compound of claim 3 wherein the moiety forms an ester or cyclic diester with the phosphate, thiophosphate, phosphonate, or phosphoamidate group.

5. The compound of claim 4 wherein the moiety comprises a pivaloyl group or an S-acyl-thioethyl group.

6. The compound of claim 1 having the structure of Formula 2




*Formula 2*

wherein $R^1$ is H, OH, O(monophosphate), O(diphosphate), O(triphosphate), O(acyl), O(alkyl), O(alkenyl), S(alkyl), S(O)(alkyl), S(O)(O)(alkyl), phosphonate, $CH_2$(phosphonate), or halogen, and where $R_1$ comprises a phosphate or phosphonate, the phosphate or phosphonate is optionally stabilized or masked;

$R^2$, $R^3$, $R^4$, $R^7$ and $R^8$ are independently H, OH, O(acyl), O(alkyl), O(alkenyl), O(alkynyl), S(alkyl), S(O)(alkyl), S(O)(O)(alkyl), alkyl, alkenyl, alkynyl, cyano, $CF_3$, $CHF_2$, $CH_2F$, $CH_2OH$, $CH_2O$(alkyl), -CH=CHBr, $-CH_2CH_2Br$, C(O)O(alkyl), halogen, azido, $NO_2$, $NH_2$, NH(alkyl), -NH(acyl), -N(alkyl)$_2$, or N(acyl)$_2$;

$R^5$ and $R^6$ are independently H, OH, O(acyl), O(alkyl), O(alkenyl), S(alkyl), S(O)(alkyl), S(O)(O)(alkyl), alkyl, alkenyl, alkynyl, cyano, $CF_3$, $CHF_2$, $CH_2F$, $CH_2OH$, $CH_2O$(alkyl), -CH=CHBr, $-CH_2CH_2Br$, C(O)O(alkyl), halogen, azido, $NO_2$, $NH_2$, NH(alkyl), NH(acyl), N(alkyl)$_2$, N(acyl)$_2$;

wherein E is O, S, $SO_2$, C(=$CH_2$), $NR_6$, or $CH_2$;

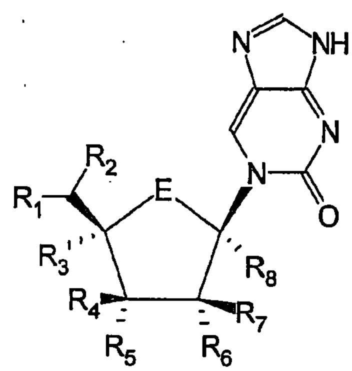
optionally one of $R_4$ and $R_5$ is null, then the other of $R_4$ and $R_5$ is coupled to the sugar via a double bond;

optionally one of $R_6$ and $R_7$ is null, then the other of $R_6$ and $R_7$ is coupled to the sugar via a double bond; and

optionally $R_5$ and $R_6$ are null, then the C2'-atom and C3'-atom of the sugar are coupled to each other via a double bond.

7. The compound of claim 6 having an L-configuration.

8. The compound of claim 1 having the structure of Formula 3



*Formula 3*

wherein $R^1$ is H, OH, O(monophosphate), O(diphosphate), O(triphosphate), O(acyl), O(alkyl), O(alkenyl), S(alkyl), S(O)(alkyl), S(O)(O)(alkyl), phosphonate, $CH_2$(phosphonate), or halogen, and where $R_1$ comprises a phosphate or phosphonate, the phosphate or phosphonate is optionally stabilized or masked;

$R^2$, $R^3$, $R^4$, $R^7$ and $R^8$ are independently H, OH, O(acyl), O(alkyl), O(alkenyl), O(alkynyl), S(alkyl), S(O)(alkyl), S(O)(O)(alkyl), alkyl, alkenyl, alkynyl, cyano, $CF_3$, $CHF_2$, $CH_2F$, $CH_2OH$, $CH_2O$(alkyl), -CH=CHBr, $-CH_2CH_2Br$, C(O)O(alkyl), halogen, azido, $NO_2$, $NH_2$, NH(alkyl), -NH(acyl), $-N(alkyl)_2$, or $N(acyl)_2$;

$R^5$ and $R^6$ are independently H, OH, O(acyl), O(alkyl), O(alkenyl), S(alkyl), S(O)(alkyl), S(O)(O)(alkyl), alkyl, alkenyl, alkynyl, cyano, $CF_3$, $CHF_2$,

$CH_2F$, $CH_2OH$, $CH_2O$(alkyl), -CH=CHBr, -$CH_2CH_2Br$, C(O)O(alkyl), halogen, azido, $NO_2$, $NH_2$, NH(alkyl), NH(acyl), N(alkyl)$_2$, N(acyl)$_2$;

wherein E is O, S, $SO_2$, C(=$CH_2$), $NR_6$, or $CH_2$;

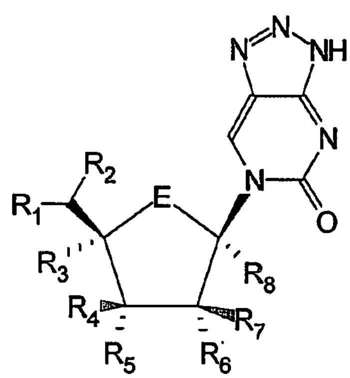
optionally one of $R_4$ and $R_5$ is null, then the other of $R_4$ and $R_5$ is coupled to the sugar via a double bond;

optionally one of $R_6$ and $R_7$ is null, then the other of $R_6$ and $R_7$ is coupled to the sugar via a double bond; and

optionally $R_5$ and $R_6$ are null, then the C2'-atom and C3'-atom of the sugar are coupled to each other via a double bond.

9. The compound of claim 8 having an L-configuration.

10. The compound of claim 1 having the structure of Formula 4



*Formula 4*

wherein $R^1$ is H, OH, O(monophosphate), O(diphosphate), O(triphosphate), O(acyl), O(alkyl), O(alkenyl), S(alkyl), S(O)(alkyl), S(O)(O)(alkyl), phosphonate, $CH_2$(phosphonate), or halogen, and where $R_1$ comprises a phosphate or phosphonate, the phosphate or phosphonate is optionally stabilized or masked;

$R^2$, $R^3$, $R^4$, $R^7$ and $R^8$ are independently H, OH, O(acyl), O(alkyl), O(alkenyl), O(alkynyl), S(alkyl), S(O)(alkyl), S(O)(O)(alkyl), alkyl, alkenyl, alkynyl, cyano, $CF_3$, $CHF_2$, $CH_2F$, $CH_2OH$, $CH_2O$(alkyl), -CH=CHBr, -$CH_2CH_2Br$,

C(O)O(alkyl), halogen, azido, $NO_2$, $NH_2$, NH(alkyl), -NH(acyl), -$N(alkyl)_2$, or $N(acyl)_2$;

$R^5$ and $R^6$ are independently H, OH, O(acyl), O(alkyl), O(alkenyl), S(alkyl), S(O)(alkyl), S(O)(O)(alkyl), alkyl, alkenyl, alkynyl, cyano, $CF_3$, $CHF_2$, $CH_2F$, $CH_2OH$, $CH_2O$(alkyl), -CH=CHBr, -$CH_2CH_2Br$, C(O)O(alkyl), halogen, azido, $NO_2$, $NH_2$, NH(alkyl), NH(acyl), $N(alkyl)_2$, $N(acyl)_2$;

wherein E is O, S, $SO_2$, C(=$CH_2$), $NR_6$, or $CH_2$;

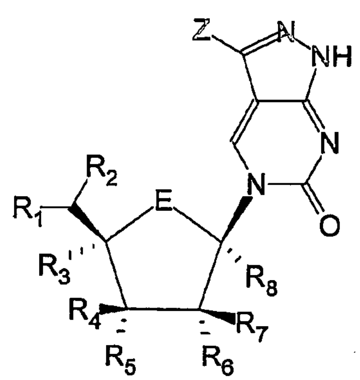
optionally one of $R_4$ and $R_5$ is null, then the other of $R_4$ and $R_5$ is coupled to the sugar via a double bond;

optionally one of $R_6$ and $R_7$ is null, then the other of $R_6$ and $R_7$ is coupled to the sugar via a double bond; and

optionally $R_5$ and $R_6$ are null, then the C2'-atom and C3'-atom of the sugar are coupled to each other via a double bond.

11. The compound of claim 10 having an L-configuration.

12. The compound of claim 1 having the structure of Formula 5




*Formula 5*

wherein $R^1$ is H, OH, O(monophosphate), O(diphosphate), O(triphosphate), O(acyl), O(alkyl), O(alkenyl), S(alkyl), S(O)(alkyl), S(O)(O)(alkyl), phosphonate, $CH_2$(phosphonate), or halogen, and where $R_1$ comprises a phosphate or

phosphonate, the phosphate or phosphonate is optionally stabilized or masked;

$R^2$, $R^3$, $R^4$, $R^7$ and $R^8$ are independently H, OH, O(acyl), O(alkyl), O(alkenyl), O(alkynyl), S(alkyl), S(O)(alkyl), S(O)(O)(alkyl), alkyl, alkenyl, alkynyl, cyano, $CF_3$, $CHF_2$, $CH_2F$, $CH_2OH$, $CH_2O$(alkyl), -CH=CHBr, $-CH_2CH_2Br$, C(O)O(alkyl), halogen, azido, $NO_2$, $NH_2$, NH(alkyl), -NH(acyl), -N(alkyl)$_2$, or N(acyl)$_2$;

$R^5$ and $R^6$ are independently H, OH, O(acyl), O(alkyl), O(alkenyl), S(alkyl), S(O)(alkyl), S(O)(O)(alkyl), alkyl, alkenyl, alkynyl, cyano, $CF_3$, $CHF_2$, $CH_2F$, $CH_2OH$, $CH_2O$(alkyl), -CH=CHBr, $-CH_2CH_2Br$, C(O)O(alkyl), halogen, azido, $NO_2$, $NH_2$, NH(alkyl), NH(acyl), N(alkyl)$_2$, N(acyl)$_2$;

wherein E is O, S, $SO_2$, C(=$CH_2$), $NR_6$, or $CH_2$;

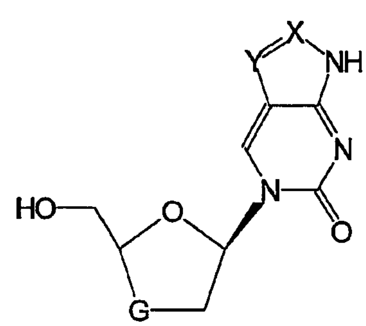
optionally one of $R_4$ and $R_5$ is null, then the other of $R_4$ and $R_5$ is coupled to the sugar via a double bond;

optionally one of $R_6$ and $R_7$ is null, then the other of $R_6$ and $R_7$ is coupled to the sugar via a double bond; and

optionally $R_5$ and $R_6$ are null, then the C2'-atom and C3'-atom of the sugar are coupled to each other via a double bond.

13. The compound of claim 12 having an L-configuration.

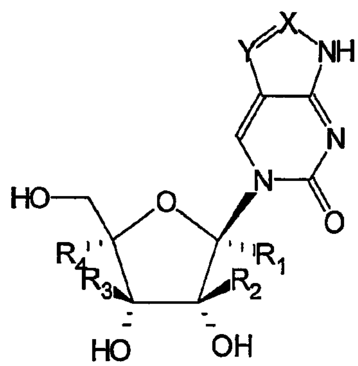
14. The compound of claim 1 having a structure according to Formula 6:




*Formula 6*

wherein G is S, O $CH_2$, or CHOH, and wherein the compound is in a D- or an L-configuration.

15. The compound of claim 1 having a structure according to Formula 7:

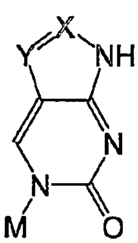


*Formula 7*

wherein one of $R_1$, $R_2$, $R_3$, and $R_4$ is alkyl, and the remaining of $R_1$, $R_2$, $R_3$, and $R_4$ are H; and

wherein the compound is in an L- or D-configuration.

16. A pharmaceutical composition comprising a compound according to Formula 1



*Formula 1*

wherein -X=Y- is -N=N-, -CH=N-, -N=CZ- or -CH=CZ-, wherein Z is H, halogen, or alkyl, and wherein M is a sugar or sugar analog;

wherein the compound has a D-configuration or an L-configuration; and

wherein the compound is present at a concentration effective to inhibit propagation of a virus in a patient to which the composition is administered.

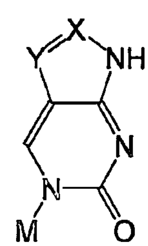
17. The pharmaceutical composition of claim 16 wherein the compound further comprises a phosphate, thiophosphate, phosphonate, or phosphoamidate group covalently coupled to the sugar or sugar analog.

18. The pharmaceutical composition of claim 17 further comprising a moiety that increases selectivity of the compound to a target cell, and wherein at least part of the moiety is removed from the compound in the target cell.

19. The pharmaceutical composition of claim 18 wherein the moiety forms an ester or cyclic diester with a phosphate, thiophosphate, phosphonate, or phosphoamidate group.

20. The pharmaceutical composition of claim 16 wherein the virus is an HCV virus, an HIV virus, an RSV virus, an influenza virus, or a an HBV virus.

21. A method of treating a viral infection in a patient comprising administering a compound according to Formula 1 to the patient at a dosage effective to reduce viral propagation in the patient



*Formula 1*

wherein -X=Y- is -N=N-, -CH=N-, -N=CZ- or -CH=CZ-, wherein Z is H, halogen, or alkyl, and wherein M is a sugar or sugar analog; and

wherein the compound has a D-configuration or an L-configuration.

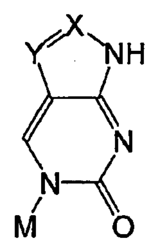
22. The method of claim 21 wherein the virus is an HCV virus.

23. The method of claim 21 wherein the virus is an HIV virus.

24. The method of claim 21 wherein the virus is an HBV virus.

25. A method of inhibiting a viral polymerase comprising:

presenting the viral polymerase with a compound according to Formula 1 at a concentration effective to inhibit the viral polymerase



*Formula 1*

wherein -X=Y- is -N=N-, -CH=N-, -N=CZ- or -CH=CZ-, wherein Z is H, halogen, or alkyl, and wherein M is a sugar or sugar analog; and

wherein the compound has a D-configuration or an L-configuration.

26. The method of claim 25 wherein the step of presenting further comprises conversion of a prodrug of the compound according to Formula 1 into the compound according to Formula 1.

27. The method of claim 25 wherein the step of presenting further comprises conversion of the compound according to Formula 1 into a metabolite of the compound according to Formula 1.

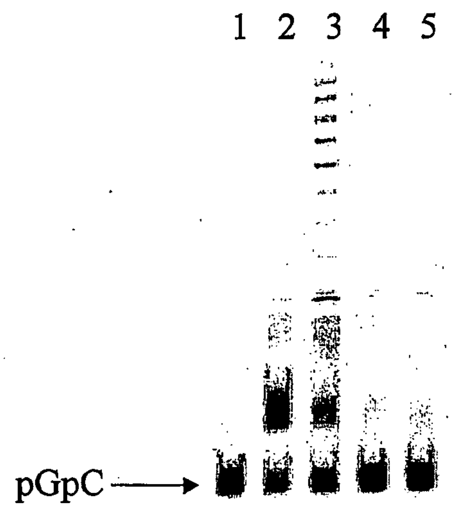



**Figure 1A**

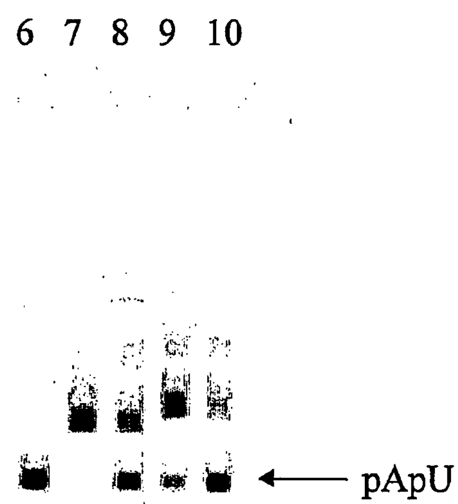



**Figure 1B**

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/06992

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : A61K 31/522, 31/519, 31/7068; C07H 19/14, 19/16, 19/20, 19/173, 19/23; C07F 9/6561; C07D 473/28
US CL : 544/244, 265, 280, 262, 254; 514/81, 263.3, 45, 48, 52, 263.23, 261.1, 265.1, 262.1; 536/26.21, 26.7

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 544/244, 265, 280, 262, 254; 514/81, 263.3, 45, 48, 52, 263.23, 261.1, 265.1, 262.1; 536/26.21, 26.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS online

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | KALMAN et al. Inactivation of Thymidylate Synthetase by a Novel Mechanism-Based Enzyme Inhibitor: 1-(B-D-2'-Deoxyribofuranosyl) 8-Azapurin-2-One 5'-Monophosphate. Biochem. Biphys. Res. Commun. 30 September 1981, Vol. 102, No. 2, pages 682-689, see figure 3, both structures. | 1-3, 10-11 |
| X | AHMED, A.F.S. Synthesis of Some Uridine and Cytidine Derivatives. Egypt. J. Pharm. Sci. 1996, Vol. 37, No. 1-6, pages 303-311, see compound B. | 1, 3, 8, 9 |
| X | HOLY, A. Preparation of 9-(B-D-Ribofuranosyl)-2-Hydroxypurine. Coll. Czech. Chem. Commun. 1979, Vol. 44, No. 9, pages 2846-2853, see II. | 1, 3, 8, 9 |
| X | FOX et al. Nucleosides. VIII. Synthesis of 5-Nitrocytidine and Related Nucleosides. J. Organic Chemistry. 1961, Vol. 26, pages 525-532, see Va, Vb, VIIIb and IVa. | 1-3, 8-11 |
| X,E | EP 1 288 313 A2 (AGILENT TECHNOLOGIES, INC.) 05 March 2003, see examples. | 1-7 |
| X | US 2002/0197618 A1 (SAMPSON) 26 December 2002, see paragraph 108, dPTP and also P-triphosphate. | 1-4, 6, 7 |

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

| | |
|---|---|
| * Special categories of cited documents: | |
| "A" document defining the general state of the art which is not considered to be of particular relevance | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "E" earlier application or patent published on or after the international filing date | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "O" document referring to an oral disclosure, use, exhibition or other means | |
| "P" document published prior to the international filing date but later than the priority date claimed | "&" document member of the same patent family |

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|---|---|
| Date of the actual completion of the international search<br>23 March 2004 (23.03.2004) | Date of mailing of the international search report<br>02 APR 2004 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450<br>Alexandria, Virginia 22313-1450<br>Facsimile No. (703) 305-3230 | Authorized officer<br>Mark L. Berch<br>Telephone No. (571) 272-1600 |

PCT/US03/06992

INTERNATIONAL SEARCH REPORT

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | LIU et al. Promoter Clearance by T7 RNA Polymerase: Initial Bubble Collapse and Transcript Dissociation Monitored by Base Analog Fluorescence. J. Biol. Chem. 25 January 2002, Vol. 277, No. 4, pages 2725-2731, see pyrrolo-dC and note attached sheet identifying the species. | 1, 6, 7 |
| X | WOO et al. G/C-modified oligodeoxynucleotides with selective complementarity: synthesis and hydbridization properties. Nucleic Acids Researcg. 1996, Vol. 24, No. 13, pages 2470-2475, see Figure 4, compound dp. | 1, 6, 7, 14 |
| X | JP 62-255499 A (TEIJIN, LTD) 07 November 1987, see translation, see Formula I; see (f) at page 6, last formula, (f) as monophosphate on page 7 in example 3, (g), as d, phosphate in example 4 and triphosphate (h) in example 5 on page 8. | |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/06992

**Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest** ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

PCT/US03/06

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING**

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-5 (in-part), 10-11and 14-27 (in-part) drawn to X-Y is first choice.

Group II, claim(s) 1-5 (in-part), 12-13, 14-17 (in-part), drawn to X-Y is second choice.

Group III, claim(s) 1-5 (in-part), 8-9 and 14-27 (in-part), drawn to X-Y is third choice.

Group IV, claim(s) 1-5 (in-part), 6-7 and 14-27 (in-part), drawn to X-Y is fourth choice.

The inventions listed as Groups I, II, III and IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Each provides its own special technical feature in the form of its own heterocyclic ring system. Group I is a triazolopyrimidinone, Group III is an imidazopyrimidone, Group II is purinone, and Group IV if a pyrrolopyrimidinone. This is the part of the molecule responsible for novelty, since the rest is just "a sugar".